

ESSENTIALITY OF ARGININE IN TOTAL
PARENTERAL NUTRITION OF THE RAT

By

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LIST OF ABBREVIATIONS

Terms

AGPA	L- α -amino- β -guanidinopropionic acid
BSA	bovine serum albumin
BW	body weight
CP	carbamoyl phosphate
CTAB	Hexadecyltrimethylammonium bromide
d-d-H ₂ O	distilled-deionized water
DAMO	2,3-butanedione monoxime
GSA	glutamate- γ -semialdehyde
i.d.	inside diameter
MOPS	3-[morpholino]propanesulfonic acid
NAG	N-acetylglutamate
o.d.	outside diameter
PAH	para-amino hippurate
P-5-C	pyrroline-5-carboxylate
q.s.	quantus suffa; bring to volume
rpm	revolutions per minute
TCA	trichloroacetic acid
TN	total nitrogen
TPN	total parenteral nutrition
TRIZMA	(Tris-(hydroxymethyl) aminomethane
UUN	urinary urea nitrogen

Enzymes and Cofactors

A	arginase
ADP	adenosine diphosphate
AL	argininosuccinate lyase
AMP	adenosine monophosphate
AS	argininosuccinate synthetase
ATP	adenosine triphosphate
CL	citrate lyase
CPS-I	carbamoyl phosphate synthetase-I
EDTA	ethylenediaminetetraacetic acid
MDH	malate dehydrogenase
NAD ⁺	nicotinamide adenine dinculeotide
NADH	reduced nicotinamide adenine dinucleotide
OAT	ornithine aminotransferase
OCT	ornithine carbamoyl transferase
PEP	phosphoenolpyruvate
U	units of activity

Units of Measure

Mw	molecular weight		
Kg	kilogram	g	grams
mg	milligram	μ g	microgram
mmol	millimole	μ mol	micromole
M	molar	mM	millimolar
mEq	milliequivalents	mOsmol	milliosmols
L	liter	dL	deciliter
mL	milliliter	μ L	microliter
d	day	hr	hour
min	minute	sec	second
cm	centimeter	mm	millimeter
μ m	micron or micrometer	nm	nanometer

Amino Acids

Ala	alanine	Leu	leucine
Arg	arginine	Lys	lysine
Asn	asparagine	Met	methionine
Asp	aspartate	Orn	ornithine
Cit	citrulline	Phe	phenylalanine
Cys	cysteine	Pro	proline
Gln	glutamine	Ser	serine
Glu	glutamate	Thr	threonine
Gly	glycine	Trp	tryptophan
His	histidine	Tyr	tyrosine
Ile	isoleucine	Val	valine
AA	amino acid	TAA	total amino acids

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Total parenteral nutrition (TPN) is a nutritional management technique for individuals who are unable to tolerate food by the oral or enteral route. A rare complication of TPN is hyperammonemia which is unrelated to organ dysfunction and there is clinical evidence that sufficient arginine in the TPN formulation alleviates and prevents recurrence of hyperammonemia. The etiology of TPN-associated hyperammonemia is unclear; it may be due to inadequate supply of arginine and/or one of its precursors to the liver or kidney for urea synthesis or to insufficient urea cycle enzyme activity.

This research established an appropriate rat TPN model for the study of arginine-related urea cycle insufficiency. Young adult rats (250 g) were maintained on TPN containing approximately 1.6, 2.5, 3.2 and 4.8% arginine after a 4-day

arginine-free infusion. The formula also contained dextrose, fat emulsion, vitamins and minerals and provided 290 mg N and 60 non-N Kcal/day. During the arginine-free period, urinary excretion of orotate increased 20-fold, urinary citrate increased 6-fold and urinary urea and total N doubled. Inclusion of arginine in the TPN formula lowered urinary orotate to control levels within 48 hours and urinary urea and total N within 96 hours with all formulas except 1.6%. The plasma urea cycle amino acids, arginine and citrulline were depressed in all TPN groups after 5 days of supplemented infusion. Regression analysis suggested an arginine requirement of 4.1% arg ($R^2=0.825$, $p<.001$) which resulted in an orotate excretion of $\leq 100 \mu\text{g/day}$. This compares with a reported need of 6-8% to prevent orotic aciduria in rats on semi-purified diets. Urea cycle enzyme activities of carbamoyl phosphate synthetase, argininosuccinate synthetase and arginase were elevated in the liver during an arginine-devoid TPN infusion relative to the enzyme activities during a 4.5% arginine-supplemented period or in control rats. Arginine supplementation resulted in increased kidney arginase activities relative to control and arginine-devoid groups. Portal and renal blood flows were elevated 1.4 and 1.3-fold, respectively, in TPN-administered rats relative to controls.

INTRODUCTION

Total parenteral nutrition (TPN) is a nutritional management technique for individuals who are unable to tolerate food by the oral or enteral route. An uncommon, but potentially fatal, complication of TPN is hyperammonemia which can be unrelated to hepatic or renal insufficiency. This type of hyperammonemia is indicative of compromised nitrogen disposal mechanisms, the etiology of which has been ascribed to an arginine deficiency in the TPN formulation. Arginine is not considered an essential amino acid for humans according to the Recommended Dietary Allowances (National Research Council, 1980), but is thought perhaps to be limiting under special circumstances of infant parenteral feeding as reported by Heird et al. (1972). Arginine requirements have not been established for TPN, nor have arginine metabolism and urea cycle activities been studied under TPN.

Nitrogen intake in excess of requirements is disposed primarily by the synthesis and urinary excretion of urea. Only the liver contains sufficient quantities of all five of the urea cycle enzymes to detoxify excess nitrogen and thus, the liver is the major site for urea synthesis. However,

the liver does not release arginine in any appreciable amounts to the systemic circulation. Although the kidney also contains all five of the urea cycle enzymes, the major role of these enzymes in the kidney is to supply arginine to peripheral tissues (Featherston et al., 1973). Although the etiology of TPN-associated hyperammonemia has been ascribed to a dietary deficiency in arginine, alterations in urea cycle enzyme activities and/or the supply of arginine precursors to the liver or kidney may also play a role and may lead to the requirement for arginine during TPN. Thus, urea cycle enzyme activities and precursor supply to the liver and kidney should be evaluated.

Adult rats have also been shown to be sensitive to arginine deficiency, which is revealed by urinary excretion of orotic acid, a metabolite which is present in abnormally high amounts and is an indicator of urea cycle insufficiency. The rat is a well established model for the study of arginine metabolism (Milner, 1985; Visek and Shoemaker, 1986) and has been used extensively as a TPN model since 1972 (Steiger et al., 1972).

The specific objectives of this research were to

1. Develop an appropriate model to assess arginine metabolism and adequacy for young adult rats on TPN.
2. Determine the adequacy of arginine for young adult rats on TPN.
3. Determine carbamoyl phosphate synthetase (CPS-I),

argininosuccinate synthetase (AS) and arginase (A) enzyme activities in the liver and argininosuccinate synthetase (AS) and arginase (A) enzyme activities in the kidney of arginine deficient and arginine sufficient rats on TPN.

4. Measure arteriovenous amino acid differences across the liver and the kidney in arginine deficient and sufficient rats on TPN.

Arginine adequacy was assessed as the ability of arginine replacement in a TPN formulation to decrease and maintain low urinary orotate excretion, an index of urea cycle sufficiency. Arginine adequacy studies yielded information on the appropriate arg/total amino acid ratio in the TPN formulation that provided the rat adequate arginine for optimal operation of the urea cycle as well as for peripheral protein synthesis. Urea cycle enzyme activities provided information on the capacity of the liver to detoxify ammonia as well as the capacity of the kidney to supply arginine to peripheral tissues during TPN. Amino acid difference data provided information on arginine utilization and arginine precursor metabolism in the from the gut, liver and kidney.

REVIEW OF THE LITERATURE

Total Parenteral Nutrition

Total parenteral nutrition (TPN) has been in clinical practice since the late 1960s. Total parenteral nutrition is a feeding technique which provides all or nearly all of the nutritional needs of an individual intravenously (Reinhardt et al., 1977). This definition of TPN differs from that of intravenous hyperalimentation which Rhoads et al. (1981) defines as the intravenous administration of nutrients in amounts substantially in excess of maintenance requirements and is therefore designed for correction of deficits.

Hyperammonemia in TPN

Total parenteral nutrition-associated hyperammonemia or excessive blood ammonia concentrations is unrelated to metabolic complications. In humans, hyperammonemia is defined as blood ammonia levels greater than 250 $\mu\text{g/dL}$ (Seashore, 1980). Total parenteral nutrition-associated hyperammonemia was at one time ascribed to the use of

protein hydrolysates made from fibrin or casein in which as much as 8 to 10% of the available nitrogen in the product was derived from free ammonia as a by-product of the protein processing method (Dudrick et al., 1972). However, hyperammonemia has also been experienced by patients receiving TPN solutions comprised of crystalline L-amino acids in which free ammonia levels of the TPN solutions are low (Heird et al., 1972). For example, ammonia levels in L-amino acid solutions range from 135-600 $\mu\text{g/dL}$ depending upon manufacturer (Heird et al., 1972; Caldwell et al., 1977; Seashore, 1980) versus 20,000-36,000 $\mu\text{g/dL}$ in 3.0% protein hydrolysates (Heird et al., 1972).

Although the etiology of TPN-associated hyperammonemia is still unclear, Johnson et al. (1972) was one of the first to demonstrate that hyperammonemia was directly proportional to the amount of protein hydrolysate infused under TPN. In this study, an infusion rate between 256 to 611 mg N/kg body weight/day (1.60-3.89 g amino acids/kg BW/day) of either fibrin or casein hydrolysate resulted in blood ammonia levels of 103 to 304 $\mu\text{g NH}_3\text{-N/dL}$ in infants between 2 and 135 days of age at the initiation of TPN (93 ± 23 $\mu\text{g NH}_3\text{-N/dL}$ is normal for the neonatal period). A supplement of 350 mg/day of arginine (2.01 mmol/day) did not improve the hyperammonemia. In contrast to this study, Heird et al. (1972) demonstrated that in their patients receiving a TPN solution of approximately 2.5 g/kg body weight/day of

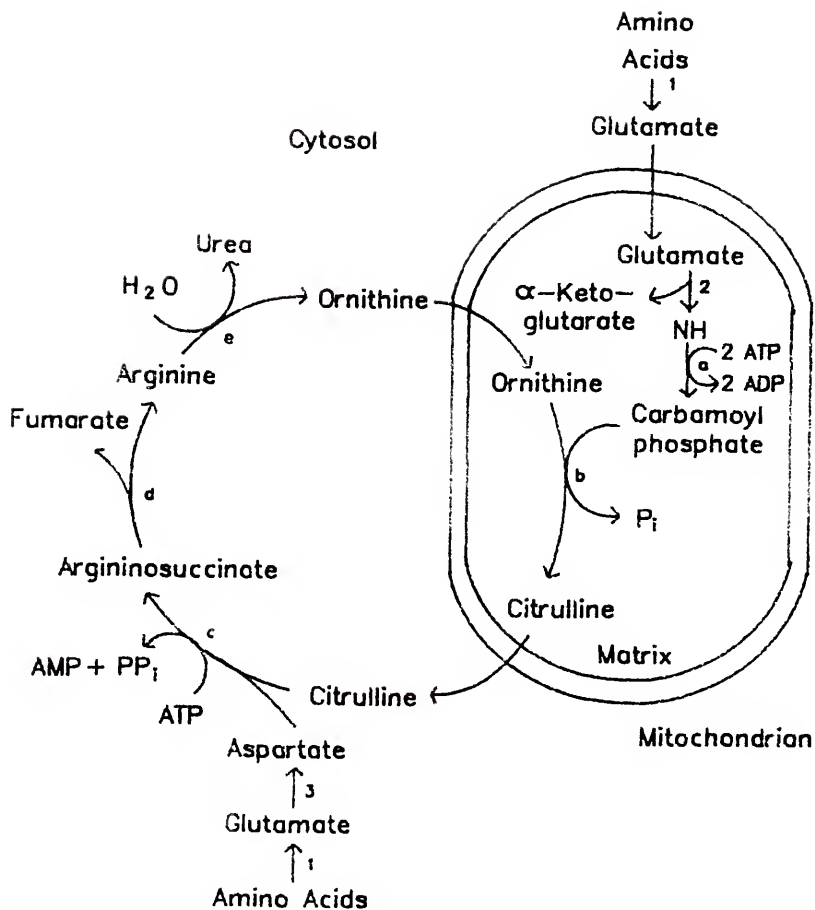
synthetic L-amino acids, hyperammonemia could be corrected within as little as 4 hours after the administration of 2.0 mmol arginine-glutamate/kg BW or 3 mmol arginine-HCl/kg BW to the daily infusate.

Role of Arginine in Prevention of Hyperammonemia

Although not specifically investigated, the ability of arginine to decrease blood ammonia levels has been attributed to the role of arginine in the urea cycle. Arginine participates directly in the urea cycle by two different modes of action. First, it is the substrate for arginase which forms urea and ornithine, a waste product and urea cycle intermediate, respectively. Secondly, arginine has been shown in vitro to have a stimulatory effect on the enzyme N-acetylglutamate synthetase (Kawamoto et al., 1982) which is independent of de novo protein synthesis (Kawamoto and Tatibana, 1983). The product of this enzyme reaction, N-acetylglutamate (NAG) is a specific positive allosteric effector for carbamoyl phosphate synthetase-I (CPS-I), the first rate limiting enzyme for urea synthesis. Low CPS-I activity may result in insufficient capacity to remove excess ammonia. Thus, a deficiency of arginine can lead to hyperammonemia by two distinct routes both of which result in decreased capacity to incorporate ammonia into urea.

Urea Cycle

Figure 1 depicts the complete urea cycle which consists of five enzymes: carbamoyl phosphate synthetase-I (CPS-I), ornithine carbamoyl transferase (OCT), argininosuccinate synthetase (AS), argininosuccinate lyase (AL) and arginase (A). All five of these enzymes are present in the liver and are compartmentalized between the mitochondria and the cytosol. Carbamoyl phosphate synthetase-I and OCT are located in the mitochondria and catalyze the conversion of NH_4^+ and HCO_3^- to carbamoyl phosphate and ornithine and carbamoyl phosphate to citrulline, respectively. Powers-Lee et al. (1987) demonstrated that liver CPS-I and OCT are both loosely associated with the inner mitochondrial membrane which may serve as an organizing factor for the two enzymes. This finding supports the observed channeling of extramitochondrial ornithine to OCT in isolated mitochondria (Cohen et al., 1987). Citrulline is transported into the cytosol where AS catalyzes the condensation of citrulline plus aspartate to form argininosuccinate which AL cleaves to arginine and fumarate. Finally, A cleaves arginine to urea and ornithine. Urea then diffuses out of the liver via the blood and ornithine is transported into the mitochondria to initiate another round of the urea cycle. Although CPS-I is technically the committing step for the urea cycle, AS is actually considered the rate limiting step in the cycle



Enzymes

- a = carbamoyl phosphatase I
- b = ornithine carbamoyl transferase
- c = argininosuccinate synthetase
- d = argininosuccinate lyase
- e = arginase

- 1 = transamination to α -ketoglutarate
- 2 = glutamate dehydrogenase
- 3 = transamination to oxaloacetate

Figure 1. Enzymes Involved and Cellular Compartmentalization of the Urea Cycle.

because of its lower total activity compared to CPS-I (Schimke, 1962a; Briggs and Freedland, 1977; Snodgrass and Lin, 1981). Thus, the urea cycle functions in the liver to remove two molecules of ammonia and one molecule of carbon dioxide while converting them to urea, a less toxic and more easily excreted form of nitrogen, relative to ammonia. The first molecule of ammonia arises in the mitochondria from the oxidative deamination of glutamate by glutamate dehydrogenase whereas the second molecule arises in the cytosol from aspartate via the transamination of oxaloacetate.

Urea synthesis has two regulatory mechanisms. Long-term regulation is achieved by induction of the urea cycle enzymes themselves. Urea cycle enzymes have been shown to increase in activity during conditions causing increased protein catabolism such as starvation, consumption of a high protein diet or corticosteroid treatment (Briggs and Freedland, 1977). Amino nitrogen arising from the utilization of amino acids as an energy source is excreted largely as urea (Schimke, 1962a). There is a differential effect on the excretion of urea during either consumption of a protein-free diet or starvation even though both result in extensive loss of total liver protein and other tissue components. During prolonged fasting (7 days in rats), energy is derived from body protein stores and results in increased urea excretion. During consumption of a protein-

free diet, energy is derived from dietary carbohydrate and fat with a resultant decrease in urea excretion. Schimke (1962b) showed that consumption of a protein-free diet decreased total urea cycle enzyme activity 20-50% whereas fasting increased total urea cycle enzyme activity by 300%. Muramatsu and Ashida (1962) showed that diets of differing protein quality fed at the same rate resulted in higher liver arginase activities in rats given higher quality protein diet, but was accompanied by a lower urinary urea excretion. Abrupt change from a 30% protein diet to either a 15 or 60% protein diet adaptively altered urea cycle enzymes within 8 days. Animals changed to the 60% protein diet showed dramatic increases in CPS-I, OCT AND AL within 4 days, and reached a maximum increase by 8 days. Arginase specific activity increased more slowly than the other enzymes, but also reached maximum increase by 8 days. These four enzymes declined in specific activity to approximately one-half of the peak maxima by the end of the 28 day study. Decreasing the protein content to 15% showed an opposite effect. Carbamoyl phosphate synthetase-I, OCT and AL showed a slight decline in specific activity which leveled out by 8 days. Arginase specific activity showed a slight but steady decline over the entire 28 day study period. Schimke (1962a) concluded that the quantitative relationship between the hepatic content of urea cycle enzymes and daily intake of protein suggest that the rate of urea synthesis is

increased or decreased by alterations in the content of the enzymes rather than by changes in substrate concentration. Although an increase requirement for urea synthesis could presumably be met most economically for the organism by increasing the activity of only that enzyme in least abundance (AS), these data show there is a comparable increase in all urea cycle enzymes. Snodgrass and Lin (1987) recently reconfirmed increased activities in the first four urea cycle enzymes in livers of rats fed arginine-deficient diets. However, cultured rat liver cells or hepatoma cells cultured as a monolayer in arginine-deficient medium did not show higher activities for these first four enzymes. The authors postulated that the arginine requirement may be very low in the resting cells of the monolayer and may be provided by proteolysis which occurs normally. Boyce et al. (1986) showed that the human AS gene is controlled by a 5' flanking sequence promoter region which is controlled by an arginine-mediated repression mechanism. It is not known if arginine itself or a repressor protein made on another gene under the influence of arginine binds to the promoter region. CPS-I, OCT and AL expression may have similar arginine-mediated repression controlled promoter regions.

Short-term regulation of urea synthesis appears to be mediated by mitochondrial concentration of NAG.

N-acetylglutamate acts as a positive allosteric effector that influences the monomer-dimer association-dissociation of CPS-I (Cohen, 1981). Stewart and Walser (1980) measured liver concentration of NAG and CPS-I activity after intraperitoneal administration of amino acids in large doses. Blood alpha amino nitrogen rose immediately after injection, peaked at 15 minutes, then dropped rapidly. The activity of CPS-I in intact mitochondria increased rapidly after injection and peaked at 30 minutes post-injection. The maximum activity attained was 40 nmol/min/mg mitochondrial protein, in contrast to a pre-injection activity of 9 nmol/min/mg mitochondrial protein. The increase in CPS-I was directly proportional to the increase of mitochondrial NAG concentration which in turn was dependent on the amino acid load injected. Both NAG concentration and CPS-I activity were maximized at a load of 3 g amino acids/kg BW.

Glucagon, a gluconeogenic hormone, has been shown to increase intramitochondrial concentrations of NAG (Hensgens et al., 1980). Citrulline production in isolated hepatic mitochondria from glucagon-injected rats was equivalent to control hepatic mitochondria incubated with NAG. Thus, the conclusion was that glucagon-induced urea production is mediated by the increased intramitochondrial NAG concentration, which in turn increased citrulline synthesis.

Thus, glucagon may be important in the cellular coordination of proteolysis and urea synthesis.

Source and Fate of Arginine, Ornithine and Citrulline

Arginine has two important physiological roles: 1) as a urea cycle intermediate for the disposal of ammonia and 2) as a constituent of proteins. Dietary arginine is not considered an essential amino acid in adult man (Rose et al., 1954, National Research Council, 1980), dog (Rose and Rice, 1939) or rat (Wolf and Corley, 1939). Nonessentiality of arginine for these adult mammals was determined by nitrogen balance and absence of weight loss while consuming an arginine devoid diet. Thus it is assumed that endogenous synthesis of arginine by these adult mammals adequately meets their needs. However, Kennan and Cohen (1961) presented evidence suggesting that adult rats have a limited capacity for arginine biosynthesis beyond their needs and that the urea cycle operates at near capacity for ammonia detoxification. Arginine is considered essential for optimal growth in many young animals, however. Growing dogs (Czarnecki and Baker, 1984), cats (Morris et al., 1979) and rats (Borman et al., 1946) have all been shown to require an exogenous arginine source to support optimal weight gain and/or prevent hyperammonemia (Table 1). Nonureotelic

Table 1. Growth Rate in Animals Supplemented with Arginine, Citrulline or Ornithine.

animal	Growth Rate (g/day)		
	+Arg	-Arg +Cit	-Arg+Orn
Rat ^a	4.1	4.4	1.6
Kitten ^b	24.8	N.D.	-6.5
	20.4	20.9	N.D.
Dog ^c	189.1	61.3	-11.3
Chick ^d	5.3	5.4	2.6
Rainbow Trout ^e	+++	+++	+

N.D. Not determined

^a Purified amino acid diet with arginine at 1.12% of the diet; isonitrogenous replacement of arginine with citrulline or ornithine. Milner and Visek, 1975.

^b Purified amino acid diet with arginine previously demonstrated as adequate (1.16% of total AA mix); equimolar replacement of arginine with citrulline or ornithine. Morris et al., 1979.

^c Purified amino acid diet with arginine at 0.4% of diet. Isomolar replacement with citrulline or ornithine. Czarnecki and Baker, 1984.

^d 1% supplement of arginine, citrulline or ornithine to basal diet which contained 0.74% arginine. Klose and Almquist, 1940.

^e Equimolar replacement of the 1.2% arginine supplement which is in addition to the arginine present in the 29% casein basal diet. Actual growth rate not given, but orn is significant at $p < .05$. Chiu et al., 1986.

animals such as chicks (Klose and Almquist, 1940) and rainbow trout (Chiu et al., 1986) have also been shown to require exogenous arginine to support optimal weight gain (Table 1). Arginine acts anaplerotically in the urea cycle and logically, either citrulline or ornithine should be able to substitute for arginine in this capacity. It has been shown in the rat (Milner and Visek, 1975), kitten (Morris et al., 1979) and dog (Czarnecki and Baker, 1984) that both citrulline and ornithine can substitute for arginine in its ability to prevent or alleviate hyperammonemia. Hoogenraad et al. (1985) provided evidence that 105 g Wistar rats fed for 10 days a 30% L-amino acid diet containing an equimolar replacement of ornithine for arginine had an equivalent growth rate compared to rats on the control diet containing 5.7% arginine of the total amino acids. The ability of ornithine to replace arginine in promoting adequate growth in young rats has not been corroborated by other investigators however, and it is generally accepted that only citrulline can substitute for arginine in providing an equivalent growth rate in some, but not all, species. In a growing animal, increased protein synthesis for lean body tissues comprises a large percentage of the increased weight gain. In an adult animal, however, weight gain reflects a greater deposition of fat stores and protein synthesis is required only for maintenance or repair.

Table 2. Tissue Distribution of Urea Cycle Enzyme Activities.

Tissue	Activity (umole/hour/g wet tissue)					Ref.
	CPS-I	OCT	AS	AL	A	
Liver	340	13,400	175	269	35,000[40,140]	a,d
Kidney	4.43	78	12	43.2	2240[2,520]	b,d
Brain	4	0	3	6.9	105[48]	b,d
Pancreas	3	2,[0.29]			{1,440}	c,e,d
Spleen	0	0,{<0.22}			[0]	c,d,e
Lung					150	d
Thymus					54	d
Skeletal Muscle		[<0.22]			0	d,e
Adrenal		[0.67]			0	d,e
Blood					0	d
Testicle		0.32				e
Intestinal Mucosa		2.60			[4,560]	e,d

Value in [] is second reference; value in {} is third reference.

a. Schimke, 1962.

b. Ratner, 1976.

c. Tamir and Ratner, 1963.

d. Greengard et al., 1970.

e. Raijman, 1974.

Arginine is the only urea cycle intermediate which is naturally found in dietary sources, as citrulline and ornithine are not incorporated into proteins. The only known endogenous source of arginine is its biosynthesis utilizing the urea cycle enzymes (Windmueller and Spaeth, 1981). Table 2 lists the tissue distribution for the urea cycle enzymes. Liver is the only tissue known to contain adequate quantities of all five of the urea cycle enzymes to effectively convert ammonia to urea as a nitrogen disposal organ. As can be seen in Table 2, kidney is the only extrahepatic tissue which also contains the full complement of urea cycle enzymes. However, general agreement is that the kidney does not significantly, if at all, contribute to ammonia disposal via urea synthesis. Only those tissues with sufficient AS and AL activities can synthesize arginine from citrulline and only those tissues containing sufficient CPS-I and OCT activities can synthesize arginine from ornithine. Thus, while the kidney has sufficient capacity to synthesize arginine starting with citrulline, it has limited capacity to synthesize arginine starting from ornithine due to its low CPS-I and OCT activity.

Rogers et al. (1972) and Featherston et al. (1973) have both shown the significant role the kidney plays in supplying arginine for both hepatic and extrahepatic protein synthesis. Skeletal muscle contains 13.4% arginine making it the most abundant amino acid in that tissue (Souba et

al., 1985b). Radioisotopic studies using either guanidino- ^{14}C arginine or ureido- ^{14}C citrulline at two levels of dietary protein consumption (8% or 80% casein) indicated that isotope incorporation into TCA-precipitable proteins in liver, muscle, spleen and kidney differed depending on dietary protein level and isotope source (Rogers et al., 1972). The label from either source is retained to a greater extent in the tissue proteins of the lower dietary protein group. Label incorporation into tissue proteins from ^{14}C -citrulline was greater in all tissues at the 80% dietary protein consumption level; however, it was only slightly greater in the liver. At the lower protein consumption level, incorporation into the kidney was over three times greater from citrulline than from arginine. It was, however, nearly equal in muscle and spleen and was 1.5 times lower in the liver. Thus, these data indicate that dietary arginine is incorporated into peripheral proteins dependent upon the protein level in the diet and that citrulline is an important precursor to arginine for subsequent protein synthesis both in the liver as well as for extrahepatic tissues. In a subsequent study, Featherston et al. (1973) showed that there was little or no label incorporation into muscle from anephretic animals from ^{14}C -citrulline, thus confirming the role of the kidney in arginine synthesis from citrulline for extrahepatic protein synthesis.

Apart from urea and protein synthesis, arginine is utilized for the biosynthesis of creatine and polyamines. Creatine acts as an ATP regeneration mechanism in the skeletal muscle by accepting the high energy phosphate from ATP in times of muscle quiescence and subsequently transferring that high energy phosphate to ADP to regenerate ATP during muscle contraction. In creatine biosynthesis, arginine transfers its guanidino group to glycine to form the intermediate guanidoacetate which is subsequently methylated to form creatine. Ornithine is formed as a product from the guanidino transfer. Arginine is purported to be the source of ornithine in polyamine biosynthesis via the action of arginase (Tabor and Tabor, 1984; Pegg, 1986).

Ornithine can be synthesized in any tissue containing arginase (Table 2). It is also synthesized as a by-product of guanidino-group transfers such as in the guanidoacetate intermediate for creatine synthesis. Ornithine has also been shown to be synthesized in the small intestine from glutamate; the reaction is presumably catalyzed by the enzyme ornithine aminotransferase, OAT (Henslee and Jones, 1982). Ornithine aminotransferase catalyzes the reversible reaction of ornithine plus α -ketoglutarate to glutamate- δ -semialdehyde (GSA) plus glutamate with the in vivo equilibrium thought to favor glutamate formation. Although there was no direct evidence for conversion of glutamate to ornithine by OAT, Henslee and Jones (1982) showed

incorporation of ^{14}C into ornithine from radiolabeled glutamate by an OAT enriched mitochondrial preparation. Rogers and Phang (1985) measured pyrroline-5-carboxylate synthase (P-5-C synthase) activity in rat small intestine and further substantiated the evidence for a biosynthetic pathway of ornithine synthesis from glutamate. The postulated glutamate to ornithine pathway involves P-5-C synthase catalyzed conversion of glutamate to GSA which in turn becomes the substrate for OAT. Ornithine is produced by the reversible OAT reaction.

Some evidence has been presented indicating that ornithine may be synthesized from glutamine. Lund and Wiggins (1986) presented evidence indicating that during urea synthesis, ornithine and NAG concentrations increase in isolated hepatocytes when incubated with glutamine. The pathway postulated for ornithine synthesis from glutamine in rat liver is similar to the bacterial system in which there is sequential conversion of N-acetylglutamate phosphate to N-acetylglutamate semialdehyde to N-acetylornithine to ornithine. This is only a postulation as the enzymes involved in this conversion have not been identified in rat liver. It is also postulated that glutamine is degraded by a liver specific, allosterically controlled glutaminase in this conversion of glutamine to ornithine.

Ornithine is of course utilized in the urea cycle as the immediate precursor to citrulline. Ornithine is also

utilized in polyamine biosynthesis. The function of polyamines is still obscure. However, there is agreement that they are essential for cell growth (Pegg, 1986) and are most ubiquitous in actively dividing or metabolizing cells and are associated with protein synthesis (Tabor and Tabor, 1984). Ornithine decarboxylase is the committing step for polyamine biosynthesis and catalyzes the decarboxylation of ornithine to yield putrescine (1,4-diaminobutane), the first in a series of three polyamines. Although ornithine is consumed at the committing step, there appears to be substantial interconversion between the three polyamines, putrescine, spermidine and spermine catalyzed by spermidine or spermine N'-acetyltransferase, respectively (Pegg, 1986); thus, the true ornithine demand for polyamine synthesis is unknown.

Endogenous citrulline is derived from ornithine and carbamoyl phosphate condensation catalyzed by OCT. Only those tissues with adequate CPS-I and OCT activity have the capacity to synthesize citrulline from ornithine. Thus the liver and small intestine are the two sources of citrulline biosynthesis. Work by Drotman and Freedland (1972) using perfused isolated rat liver indicated that with excess ammonia and ornithine, there was a net export of citrulline. This led to the hypothesis that the liver supplied citrulline for renal synthesis of arginine for extrahepatic tissues. This hypothesis has been refuted by a series of

experiments indicating that the small intestine is the source for citrulline (Windmueller and Spaeth, 1974, 1978, 1980, 1981). Windmueller and Spaeth (1974) provided evidence from isolated rat small intestine that citrulline and ornithine are released into the venous blood from U-¹⁴C-glutamine uptake which accounted for 6% and 0.3% of the labeled carbon, respectively. Calculating the percent release from glutamine-N, approximately 34% was released as citrulline. In a subsequent study, Windmueller and Spaeth (1978) presented data showing that 4.7% and 1.3% of the carbon backbone of citrulline and ornithine, respectively, are derived from glutamine uptake by the small intestine in vivo. Nitrogen extraction from glutamine uptake by perfused intact small intestine was shown to be approximately 28% for citrulline and 1% for ornithine (Windmueller and Spaeth, 1980). In a subsequent study, they determined that the kidney extracted from circulating blood approximately 84% of the intestinally-released citrulline and returned approximately 75% of that citrulline as arginine (Windmueller and Spaeth, 1981). Hoogenraad, et al. (1985) demonstrated that selective inhibition of intestinal OCT of young rats on an arg-deficient diet resulted in a rapid and complete inhibition of growth. Additionally, serum citrulline decreased nearly 2-fold, arg decreased 4.6-fold whereas ornithine increased 2.7-fold. This evidence demonstrates the role intestinally derived citrulline has in

maintaining arginine homeostasis. Liver and intestinal OCT appear to have different regulatory mechanisms which are apparently related to the respective physiological roles. Wraight et al. (1985) fed rats either a 15% or 60% protein diet after adapting the animals to a 30% protein diet. After 7 days of the experimental diet, liver OCT activity was increased; however, intestinal OCT activity was moderately but statistically decreased in the 60% protein group relative to the 15% protein group. The authors postulated that liver OCT activity was increased in response to an increased demand for nitrogen disposal whereas the decreased intestinal OCT activity was in response to a decreased requirement to provide citrulline precursor to maintain arg homeostasis. Windmueller and Spaeth (1981) provided circumstantial evidence for an extraintestinal, extrahepatic citrulline source. This was demonstrated by the absence of circulating blood citrulline depletion when the intestine alone or the intestine plus an additional organ (spleen, stomach, liver or pancreas) was excluded from the circulation. Small intestinal synthesis of citrulline from glutamine could be critically compromised for the TPN patient due to the decreased circulating glutamine as a result of the underlying conditions indicating the necessity for TPN (Souba, 1985). Hughes and Dowling (1980) demonstrated marked hypoplasia (denoted by decreased mucosal mass) and hypofunction (denoted by decreased histologically

measured mucosal thickness) within 3 days of parenteral feeding of rats. Unfortunately, it is not known if this hypoplasia and hypofunction is a result of decreased exogenous nutrition, decreased intestinal blood supply including a decrease of its normal respiratory fuels, or alteration in hormonal stimulation of the intestinal tract. Thus arginine homeostasis may be compromised in the TPN administered animal or man since exogenous nutrition to the gut is eliminated.

The fate of citrulline is for arginine biosynthesis by the two step pathway utilizing AS and AL. The two tissues containing adequate amounts of these two enzymes are the liver and kidney. Windmueller and Spaeth (1981) provided evidence that citrulline is not sequestered from the arterial supply by the liver. Their radiolabeled studies substantiated earlier arteriovenous amino acid difference evidence that the liver does not take up citrulline or ornithine (Coulson and Hernandez, 1968). Windmueller and Spaeth (1981) determined that 86% of the citrulline released by the small intestine is taken up by the kidney and that 75% of that is returned to the venous supply as arginine.

Orotate as Arginine Deficiency Indicator

Milner and Visek (1973) were among the first to provide experimental evidence that feeding rats an arginine-

deficient diet leads to increased urinary orotic acid excretion. Since that report, many reports on the positive correlation between increased urinary orotate excretion and arginine deficiency and/or ammonia toxicity have been made. The hypothesis for increased orotate synthesis is that carbamoyl phosphate (CP) is synthesized in excess of its capacity to become incorporated into citrulline; hence, CP is exported into the cytosol where it is utilized as the substrate for aspartate carbamoyl transferase and ultimately synthesized into orotate (Visek and Shoemaker, 1986). Orotate, a precursor for pyrimidines, is synthesized in excess of pyrimidine synthesis requirements and thus is excreted in the urine. The correlation between orotic aciduria and arginine deficiency and/or ammonia toxicity is well documented. In the more recent reviews (Milner, 1985; Visek and Shoemaker, 1986; Zieve, 1986), orotic aciduria is suggested as a biochemical index for arginine deficiency or ammonia toxicity.

However, the explanation for orotic aciduria is not accepted by all researchers. Barbul (1986), does not agree that the hypothesis adequately accounts for the involvement of the cytosolic enzyme, carbamoyl phosphate synthetase-II which synthesizes CP for pyrimidine biosynthesis. Barbul does not agree that the published evidence supports the hypothesis of a metabolic shunting of ammonia from one carbamoyl phosphate synthetase system to the other (CPS-I to

CPS-II). However, Natale and Tremblay (1969, 1974) and Tremblay et al. (1977) have presented substantial evidence that CP derived from CPS-I is indeed incorporated into orotate and that incorporation is substantial even under physiological conditions. Natale and Tremblay (1969) demonstrated in isolated rat liver mitochondria that carbamoyl phosphate is freely permeable to the mitochondrial membrane. In a subsequent study Natale and Tremblay (1974) demonstrated under optimal conditions for citrulline synthesis, approximately one-half of the carbamoyl phosphate synthesized was exported to the extramitochondrial medium. Tremblay et al. (1977) showed in liver slice preparations that over 80% of the $\text{NaH}^{14}\text{CO}_3$ incorporated into orotic acid at physiological levels of ammonia and ornithine was ammonia dependent rather than glutamine dependent. They demonstrated that 5 mM ammonia did not stimulate synthesis of glutamine in the liver slice reaction medium. Additionally, glutamine added to liver slice preparations stimulated the incorporation of $\text{NaH}^{14}\text{CO}_3$ into orotic acid; however, this stimulation could be accounted for by the generation of ammonia by glutaminase and was blocked by the addition of ornithine. These authors concluded that CPS-I is the major source of CP in the biosynthesis of hepatic orotate under physiological conditions as well as ammonia intoxication. Tremblay et al. (1977) also demonstrated that tissues with low or no CPS-I activities such as kidney

and spleen were not stimulated to produce ^{14}C -orotate from labeled bicarbonate under the same conditions in which liver slices produced nearly 20 times the normal amount. Arashima and Matsuda (1972, as referenced by Tremblay et al., 1977) documented a clinical case in which an infant with only 13% CPS-I activity (compared to normal) exhibited congenital hyperammonemia without concomitant orotic aciduria.

Although orotic aciduria and hyperammonemia result in many species fed an arginine-deficient diet, there is not a similar response in adult humans orally-fed an arginine-deficient diet (Carey et al., 1987). These authors showed that urinary orotate excretion actually fell in adult humans fed an arg-deficient, isonitrogenous diet. The urinary orotate excretion returned to normal levels when the nitrogen content of the arg-deficient diet was doubled. Urinary urea and blood ammonia levels stayed within normal values and increased in response to total nitrogen load. The significant finding from this experiment was a 60% fall in postprandial plasma arginine concentration following an arginine-deficient meal. The conclusion by these authors was that the biochemical indices which are useful in animal experiments to determine arginine requirement or essentiality do not indicate that there is a requirement for arginine in orally-fed adult humans. Only in TPN patients has there been a reported incidence of a biochemical index for arginine requirement indicating that insufficient

arginine was supplied to the patient (Heird et al., 1972). The 1980 Recommended Dietary Allowances (National Research Council, 1980) reports that although arginine is not required for infants or adults, it may become limiting for humans in special circumstances and references the 1972 Heird article. Thus, only in TPN may arginine become required in humans.

Measurement of Nutrient Requirement or Adequacy

The protocol for establishing protein and amino acid requirements in the past has been based on nitrogen balance studies. Recently, however, this practice has been questioned (Millward and Rivers, 1986; Young, 1986). When fed in a meal-type fashion (versus continuous as in TPN), it is well established that protein deposition in the postprandial state followed by mobilization in the postabsorptive state occurs in the adult (Millward and Rivers, 1986). The amplitude of this circadian variation also appears dependent on the protein intake. Thus, establishment of indispensable amino acid and protein requirements must account for this dependence and variability. In an opinion article published by V.R. Young (1986), radioactive isotope tracer studies were cited which indicated that as the indispensable amino acid is gradually decreased, there are significant changes in amino acid

metabolism which appear to be linked to the physiological requirement of the host.

The level of urinary orotic acid excretion has been suggested as an indicator of arginine deficiency in the rat (Milner, 1985; Visek and Shoemaker, 1986; Carey et al., 1987). Therefore, in the rat, dietary arginine adequacy is indicated by the absence of excessive orotic aciduria. No absolute value has been assigned to the level of urinary orotic acid excretion which is considered excessive or indicative of an arginine deficiency. Hassan and Milner (1979) reported orotic acid excretion levels around 100 $\mu\text{g}/\text{d}$ in control rats weighing 180-220 g and consuming a 14% L-amino acid diet. It has been shown that orotic acid excretion can increase by as much as 49 times the control level when arginine is removed from the diet of a previously well nourished rat (Milner and Visek, 1975). It has also been shown that orotic acid excretion varies with both protein intake and ratio of arginine to total protein (Milner and Visek, 1978). At the highest protein intake (35%) and 5.22% arginine/total protein, urinary orotate excretion was approximately 500 $\mu\text{g}/\text{day}$. The same level of protein intake and 10.44% arginine/total protein decreased urinary orotate excretion to less than 200 $\mu\text{g}/\text{day}$. At 14% total protein, both 5.22% and 10.44% arginine/total protein supplementation levels yielded a urinary orotate excretion level of less than 300 $\mu\text{g}/\text{day}$. Schimke (1962a, 1962b)

provided evidence that urea cycle enzyme activities are dependent on both dietary energy and protein intake levels. In the clinical situation, energy requirements and nitrogen load are tailored to each individual patient and may be altered every few days depending upon metabolic parameters (personal communication, T.G. Baumgartner). Thus, to properly determine arginine requirements and utilization under TPN, one must account for energy supply, nitrogen load and arginine/total amino acid ratio.

MATERIALS AND METHODS

Brief descriptions of assays and methodologies are presented in this section. Detailed descriptions for dietary formulations and assay or methodological procedures are presented in Appendices I and II, respectively.

Materials and Equipment

Animals

Male Sprague-Dawley rats (Harlan Sprague Dawley, Inc., Indianapolis, IN) from 225-275 g were used throughout all experiments. The animals were individually housed in suspended stainless steel wire mesh cages, 24-hour lighting was provided and the room temperature was maintained between 70°F and 75°F. Unless otherwise noted, the animals received a lactalbumin-based semipurified control diet containing approximately 0.29 g nitrogen and 60 non-nitrogen calories per day with water provided ad-libitum during the one week adaptation period. Weight gain was monitored at least three times per week and those animals gaining 3-5 g/day were assigned to an experimental regimen.

Diets

Lactalbumin Control Diet. The lactalbumin diet was formulated to meet dietary requirements recommended by the National Research Council (1978) as well as to be isonitrogenous and isocaloric to the TPN diets. Ten kilograms of diet were formulated for each experiment according to the chart in Appendix I and mixed according to the following procedure. Lactalbumin (United States Biochemical Corp., Cleveland, OH) was first supplemented with L-arginine-HCl to provide 6.5 g arg per 100 g total lactalbumin protein. This diet will be referred to hereafter as the 'supplemented lactalbumin diet'. This supplementation level was determined to be necessary from data obtained from experiments conducted during the "Development of a TPN Model for Determination of Arginine Adequacy" phase of this work. The lactalbumin control diet used in those experiments was formulated exactly as described here except the lactalbumin was not supplemented with arginine. The appropriate amount of supplemented lactalbumin plus the remaining dry ingredients were added to the mixing bowl of a commercial mixer (Univex Mixer, Universal Industries) and thoroughly mixed for 20 minutes. The corn oil was added slowly and the entire diet was mixed an additional 20 minutes. The mixed diet was placed in a plastic-lined 5 gallon container and closed with a tight-

fitting lid. The diet was stored at 4°C and portions were removed for daily use.

TPN Diets for Arginine Supplementation. Daily nitrogen and caloric requirements were calculated based on equations developed by Tao et al. (1979) for TPN-fed rats. The amino acid formulation was also derived from data presented by Tao et al. (1979). Amino acid solutions were prepared for each experiment under a laminar flow hood located in the Food Science Building. Distilled-deionized water (d-d-H₂O) which was passed through a 0.45 micron filter (Millipore Milli-Q™ Water System, Millipore Corp., Bedford, MA) and sterilized flasks were used to prepare the amino acid solutions. L-amino acids were purchased either from Sigma Chemical Company (St. Louis, MO) or Fisher Scientific Company (Pittsburgh, PA). The base amino acid solution was warmed slightly on a heated stir plate and stirred continuously to allow dissolution of all amino acids. Each amino acid was added in the stated mixing order (Base Amino Acid Solution, Appendix I) and allowed to dissolve prior to subsequent addition. The solution was cooled and then filtered through a 0.22 micron filter (Pall Cardioplegia Plus™ Filter with Solution Spike, Pall Biomedical Products Corp., East Hills, NY) into evacuated sterilized containers (Kendall McGaw Laboratories, Inc. Irvine, CA). Arginine solutions were prepared in a similar manner; the formulation is listed in Appendix I.

TPN formulas were prepared by adding the amino acid solution, arginine solution (if necessary), dextrose, macroelements (except calcium) and sterile water for injection into appropriately sized evacuated, sterile containers (Kendall McGaw Laboratories, Inc. Irvine, CA). (Name and source of commercial solutions used to compound the TPN diets are listed in Appendix I.) The solutions were compounded under a laminar flow hood in the Pharmacy Services IV-Prep room at Shands Hospital, Gainesville, FL, sealed with parafilm, boxed in the original shipping carton and stored at 4°C until use.

Commercial solutions for IV injection of calcium gluconate, lipid, vitamins and trace elements were added daily prior to hanging the TPN bottle. The TPN bottle was allowed to come to room temperature, weighed and wrapped in foil just prior to hanging. Commercial IV administration tubing (Travenol Laboratories, Inc., Deerfield, IL) was used to deliver the TPN formulas and was replaced every two days. Care was taken to avoid contamination of the administration lines during handling.

Reagents

Except for the solutions used for TPN diets, all chemicals used were reagent grade and solutions were prepared using distilled, resin deionized (Ultrapure mixed resin, Barnstead Sybron Corp., Boston, MA) water (d-d-H₂O).

Chemicals were purchased either from Fisher Scientific Co. or Sigma Chemical Co.

Spectrophotometer

Spectrophotometric analysis were performed using a Perkin-Elmer Lambda 3A UV/VIS Spectrophotometer. Urea cycle enzyme activities for one experiment were determined using a Coleman Jr. II Spectrophotometer, Model 6/20 to avoid possible ^{14}C contamination of major laboratory equipment.

Technicon AutoAnalyzer

A Technicon AutoAnalyzer I (Technicon Instruments Corp., Chauncey, NY) was used to determine concentrations of the urinary metabolites orotic acid, urea nitrogen, total nitrogen and creatinine. A description of the AutoAnalyzer system of operation is listed in Appendix II.

Urinary Metabolite Analysis

Urine Collection and Sample Preparation

Twenty-four-hour urine collections were made under 1.0 mL of 6 N H_2SO_4 to inhibit microbial growth and maintain ammonia in an ionized state. Collections were made daily between 09:00 and 10:00 hours. Urine volume was measured, diluted to 100 mL with $\text{d-d-H}_2\text{O}$, mixed thoroughly and filtered through Whatman # 1 filter paper into 39 mL (115

mm x 23 mm) flat-bottomed disposable polypropylene tubes (Sarstedt, West Germany). Dilution of urine was made to normalize the volume of urine samples and to avoid volume calculations when determining metabolite concentrations. Prepared urine samples were frozen and stored at -20°C for analysis.

Urinary Metabolite Analysis

Urinary Urea Nitrogen. Urinary urea nitrogen (UUN) was determined according to the colorimetric Technicon AutoAnalyzer method N-1c which is a modification of the procedure described by Marsh et al. (1965). The method is based on the direct reaction of urea and diacetyl monoxime (2,3-butanedione monoxime) in the presence of ferric ion and thiosemicarbazide under acidic conditions.

Creatinine. Urinary creatinine was determined according to the colorimetric Technicon AutoAnalyzer method N-11b which is a modification of the Folin and Wu procedure as described by Oser (1965). The method is based on the chromogenic product produced by creatinine condensation with picric acid in a very alkaline solution.

Urinary Total Nitrogen. A semi-micro-Kjeldahl technique adapted from procedures reported by Ferrari (1960) and Technicon AutoAnalyzer method 31-69A was used to digest the samples and convert the nitrogenous compounds to ammonium acid sulfate. Urine samples were oxidized in a

digestion mixture consisting of sulfuric acid and catalyst. Cooled samples were diluted with d-d-H₂O to 2% H₂SO₄ just prior to ammonia determination. Ammonia was determined on the Technicon AutoAnalyzer using a modification of the Berthelot reaction in which indophenol is formed by the reaction of ammonia, phenol and hypochlorite.

Orotic Acid. A combination of the manual methods described by Stajner et al. (1968) and Rogers and Porter (1968) were adapted to run on the AutoAnalyzer. The basis of the method is that orotic acid reacts with bromine water to form 5,5'-dibromobarbituric acid. This compound is reduced to barbituric acid in the presence of 5% ascorbic acid which then reacts with p-dimethylaminobenzaldehyde (Erich's reagent) to form a yellow chromophore.

Citric Acid. Two methods were used to determine the concentration of urinary citrate. The initial method was a modification of the spectrophotometric method outlined by Camp and Farmer (1967). This method is based on the formation of pentabromoacetone from citric acid and color development with thiourea. This was a tedious method due to the extraction of the pentabromoacetone into a heptane layer and subsequent color development in the heptane extract. Thus, a second method which used citrate lyase was adopted (Nielsen, 1976). The method, used without modification, is based on the conversion of citrate to oxaloacetate and acetate by citrate lyase coupled with the conversion of

oxaloacetate to malate by the NADH-dependent enzyme malate dehydrogenase.

Amino Acid Analysis

Sample Preparation

Plasma. Whole blood samples were collected in heparinized syringes and kept on ice until all samples were collected. Samples were then placed in 15 mL conical glass tubes. Spin-Quik polypropylene beads (Omni-Tech., Inc., Santa Monica, CA) were added to the tubes to aid in plasma separation. The tubes were centrifuged (International Centrifuge Model UV, International Equipment Co., Needham, MA) for 10 min at 1500 rpm and the plasma was removed from the packed cells in 500 μ L aliquots to obtain an approximate volume. Plasma was deproteinized using 50 mg sulfosalicylic acid per mL plasma. Samples were vortexed just enough to mix the plasma and sulfosalicylic acid and then centrifuged for 10 min at 2000 rpm. The clear supernatant was removed, placed in Micro-centrifuge tubes (Fisher Scientific) and stored at 0°C until analysis. Samples were analyzed on a Beckman 119CL using a physiological column.

Hydrolysis of Lactalbumin. A lactalbumin sample from the control diet was hydrolyzed according to the procedure of Roach and Gehrke (1970). Approximately 30 mg of lactalbumin was weighed into 20 x 150 mm Kimax culture tubes

fitted with Teflon-lined caps. Hydrolysis was performed with 6 N HCl at 145°C for 2 hrs. The hydrolyzed solution was cooled to room temperature and neutralized with 6 N NaOH. The neutralized solution was adjusted to pH 2.2 ± 0.05 with concentrated HCl, centrifuged to sediment any particulate and the supernatant was stored at 0°C in Micro-centrifuge tubes until analysis. Samples were analyzed on a Beckman 119CL using a hydrolysate column.

TPN Amino Acid Solutions. An amino acid solution for the arginine-devoid TPN diet and each of the four arginine-supplemented TPN diets was made according to the formulation outlined in Appendix I. The solutions were stored in Micro-centrifuge tubes and stored at 0°C until analysis. The solutions were analyzed on a Beckman 119CL using a hydrolysate column.

Analysis

Physiological Samples. Analyses were performed using a Beckman 119CL Amino Acid Analyzer (Beckman Instruments, Palo Alto, CA) equipped with an integrator (Hewlett Packard Model 3390A data system) and a single 6 x 460 mm column packed with Beckman W-3P resin.

Hydrolysis Samples. Analyses were performed using the Beckman 119CL equipped with a 6 x 460 mm single-column packed with Beckman W-3 resin (hydrolysate column). The signal was recorded on a Hewlett Packard integrator.

Urea Cycle Enzyme Activities

Sample Preparation

Whole liver and both kidneys were excised from anesthetized animals and placed in ice cold d-d-H₂O and kept on ice until homogenization for enzyme analyses, but no longer than two hours post-excision. Approximately 0.5 g of liver or kidney tissue was homogenized for 20 sec in 19 volumes of cold water using a Polytron tissue homogenizer (Brinkmann Instruments, Inc., Westbury, NY). Liver samples were taken from a central location from both the left lateral lobe and the medial lobe. Kidney samples were obtained from both kidneys from a cross-section of a sagittal dissection excluding the inner medullary region. No further dilution of the homogenate was necessary to determine the activities of CPS-I or AS; but liver homogenate was diluted 25-fold and kidney homogenate was diluted 5-fold with a 0.5% bovine serum albumin (BSA) solution to measure arginase activity.

Urea Cycle Enzyme Analyses

The procedure used for determining the three urea cycle enzyme activities was that outlined by Nuzum and Snodgrass (1976). The composition of the three incubation mixtures is listed in Appendix II. Carbamoyl phosphate synthetase-I and argininosuccinate synthetase require the supplementary

enzymes ornithine carbamoyltransferase (OCT) and argininosuccinase (AL), respectively. These two enzymes are not readily available commercially, thus, were isolated as a crude enzyme preparation from beef liver obtained at slaughter from the Animal Science Department, University of Florida, according to the procedures outlined by Marshall and Cohen (1972) and Ratner (1970), respectively. All assays were incubated at 37°C for the specified time.

Carbamoyl Phosphate Synthetase-I. Only liver tissue was analyzed for CPS-I activity as the activity in kidney is reportedly very low. The product of the enzymatic assay, citrulline, was assayed spectrophotometrically according to the procedure outlined by Boyde and Rahmatullah (1980). Activity was expressed as μmol citrulline produced per hour per mg protein or g liver.

Argininosuccinate Synthetase. Both liver and kidney tissues were analyzed for AS activity. The product of the assay, urea, was analyzed spectrophotometrically according to the procedure outlined by Nuzum and Snodgrass (1976). Activity was expressed as μmol urea produced per hour per mg protein or g tissue.

Arginase. Liver homogenate was diluted 25-fold and kidney homogenate was diluted 5-fold with a 0.5% bovine serum albumin (BSA) solution before initiation of the assay for arginase. The product of the assay, urea, was measured according to the procedure outlined by Nuzum and Snodgrass

(1976). Activity was expressed as μmol urea produced per hour per mg protein or g tissue.

Protein Determination

Tissue homogenate samples were analyzed for protein content according to the procedure of Lowry et al. (1951) using BSA as the standard.

Miscellaneous Analytical Methods

Organ Moisture

Organ moisture was determined by freeze drying the excised, weighed and frozen tissues. Freeze drying was accomplished in a Virtis shelf-type freeze dryer (The Virtis Company, Gardner, NY) by evacuating the chamber to 30-60 μm at a temperature of 50°C. Moisture was determined after approximately 48 hrs of freeze drying time when no further loss in weight was observed.

Organ Total Nitrogen

The semi-micro-Kjeldahl method was used to determine the total nitrogen content of organs. The freeze-dried tissue was ground to a fine powder with a mortar and pestle. Digestion of the sample and subsequent determination of ammonia were described previously.

Dietary Nitrogen

Total nitrogen content was determined on unsupplemented lactalbumin, supplemented lactalbumin, supplemented lactalbumin diet and all TPN diets by the semi-micro-Kjeldahl method as described previously.

Serum Urea Nitrogen

A combination of two methods for the determination of serum urea as described by Crocker (1967) and Foster and Hochholzer (1971) was developed for routine use in our laboratory. The combined spectrophotometric method takes advantage of the diacetyl monoxime-thiosemicarbazide color development with urea in the presence of ferric ion and antipyrine in a slightly acidic solution.

Technique for TPN

Catheterization

Animals were briefly anesthetized with Halothane U.S.P. (Halocarbon Laboratories, Inc., Hackensack, NJ) and maintained with ketamine-HCl (Ketaset, Veterinary Products Bristol Laboratories, Syracuse, NY) plus 0.1% acepromazine malate, injection, 10 mg/mL (TechAmerica Group, Inc. Elwood, KS) at a dose rate of 10 mg ketamine-HCl/100 g body weight (BW) intramuscularly. Surgical tools, dressings and catheters were sterilized by autoclaving before use.

Animals were shaved on the neck above the right jugular vein and back between the dorsal scapulae. The skin was washed with 70% alcohol. A small incision (2.0-2.5 cm) was made in the neck exposing the right external jugular vein. The vein was occluded distally with 5-0 silk suture, then a venotomy was performed and a 0.020" i.d. x 0.037" o.d. Sialastic^R catheter (Dow Corning Corp, Medical Products, Midland, MI) was inserted and guided through the jugular vein into the superior vena cava for a distance of approximately 3 cm from insertion site. The tip of the catheter resided $\pm$ 2 mm from the right atrium. The catheter was secured in place proximal to the insertion site using 5-0 silk suture and one drop of cyanoacrylate resin ("super" glue). The other end of the catheter was tunneled subcutaneously and exited dorsally at the midscapular region. A plastic cup assembly was centered around the catheter and sutured to the skin of the back. The incision in the neck region was closed with 9 mm wound clips (Clay Adams, Div. of Becton Dickinson & Co., Parsippany, NJ).

Surgeries were performed using a three to four person team. One to two persons catheterized the animals. Another person tunneled the catheter to its external site, sutured the cup to the back of the neck and closed the ventral opening. The final person anesthetized the animals, prepared them for surgery, connected them to the TPN administration set-up and administered post-operative care

to the animals. Generally, a surgical resident assigned to our laboratory performed both the catheterizations and securing of the catheter and assembly cup. (Names of all residents who assisted me with this research are listed in the acknowledgements.) I anesthetized the animals, connected them to the TPN administration set-up and gave post-operative care.

Administration of Total Parenteral Nutrition

The catheter exiting dorsally from the animal was connected to a segment of medical grade 0.020" i.d. x 0.060" o.d. polyvinyl transmission line (Tygon Microbore Tubing, Norton Performance Plastics, Akron, OH) via a 22 gauge stainless steel nipple. The line was threaded through a tightly wound metal coil which was attached distally to an infusion swivel (Instech Laboratories, Inc., Plymouth Meeting, PA). The metal coil and swivel assembly were attached to the cup assembly secured to the back of the animal via a tight fitting matching cap. This system allowed the animal complete freedom to move about in the metabolic cage. The infusion swivel was attached to a segment of approximately 330 cm of 0.020" x 0.060" medical grade polyvinyl transmission line. This transmission line was attached to 0.025" i.d. silicon manifold pump tubing (Fisher Scientific) via a 20 gauge stainless steel nipple. Up to six manifold pump tubings were connected to a stream

divider with 22 gauge nipple connectors at the outlet and a 13 gauge nipple connector at the inlet. The inlet was fitted with a short piece of silicon tubing into which a 16 gauge blunt needle and hub were inserted. The TPN formula was delivered through commercial IV administration tubing (Travenol Laboratories, Inc., Deerfield, IL) and connected to one stream divider. A variable speed peristaltic cassette pump (Manostat, New York, NY) equipped to deliver TPN for up to 18 rats was used to infuse the formula. Approximately 24 hours prior to animal surgery, all formula delivery tubings and stainless steel connectors were autoclaved, hung and flow rates adjusted with sterile saline.

A 3-day post-surgery recovery period was allowed during which the animals were infused with sterile saline at approximately 15 mL/day. They were allowed to consume the supplemented lactalbumin diet in the same quantity as during the adaptation period. During the third day post-surgery, the flow rate was gradually increased to 55 mL/day. An arginine-devoid TPN solution was initiated on the fourth day and per os feeding stopped. Four days later, an arginine-supplemented solution was started and administered for an additional five days. Tap water was provided ad-libitum throughout the entire period. Rats were observed several times daily for such things as separation of catheter from transmission line, infiltration of diet into the neck area

or dislodgement of catheter. Animals which had become infiltrated or had the catheter dislodge were removed from the experiment; animals with disconnected catheters were lightly anesthetized, reconnected to the TPN transmission line and allowed to continue on the experiment.

Experimental Protocols

Arg-devoid or arg-supplemented diets and/or time periods will be referred to as -Arg or +Arg, respectively. All percentages of arg in this text will refer to the percentage expressed as g arg/100 g total amino acids (TAA). The few experiments utilizing gln will be referred to in the same manner.

Development of a TPN Model for Determining Arginine Adequacy

Four separate experiments were conducted to establish an appropriate protocol to determine arg adequacy in TPN.

An initial experiment was conducted to establish the effect of a -Arg TPN infusate on urinary metabolites and on urea cycle enzyme activities. All animals were adapted at least one week to the laboratory environment and were provided a 21% protein laboratory ration and water ad-libitum. Two groups of animals were catheterized and diet

infusion was begun as soon as the animals recovered from anesthesia. One group of animals was infused with a -Arg diet and the other was fed a +Arg diet. Diet composition is outlined in Appendix I as 'Preliminary TPN Diet'. Animals were infused for four complete days. Daily urine collections were made and metabolites measured. On the fifth day, animals were sacrificed, organs were excised and weighed and urea cycle enzyme activities measured.

A second experiment was conducted in which the TPN diet was fed orally in a 30 mL drinking bottle to the animals to establish the effect of route of administration on urinary metabolites and urea cycle enzyme activities. The animals were adapted to the laboratory environment at least one week and were provided a 21% protein laboratory ration and water ad-libitum. The TPN diet was formulated as though it would be administered intravenously. Half of the volume was given between 09:00 and 10:00 hrs and the second half was given between 17:00 and 18:00 hrs. Glutamine (gln) was incorporated into this experiment to establish preliminary data on the ability of amino acids known to supply arg precursors to replace arg in a TPN formulation. In a sequential manner, two levels of +Arg and two levels of isonitrogenous replacement of arg by gln were fed for five days, each after a 3-day -Arg period. There were three groups. A control group received an unsupplemented lactalbumin which was isonitrogenous and isocaloric to the

TPN diets. A second group received the higher level of supplementation of first 3.27% arg and then 5.38% gln. The third group received the lower level of supplementation of 0.81% arg and then 1.35% gln. Unsupplemented lactalbumin controls were pair-fed isonitrogenously throughout the entire experiment. Daily urine collections were made and urinary metabolites measured. At the termination of the +gln period, animals were sacrificed, organs excised and weighed and urea cycle enzyme activities measured.

The next two experiments were parenteral infusion studies using the same design as the oral feeding study.

Establishment of Arginine Adequacy in TPN

Rats were adapted to the laboratory for one week and fed the supplemented lactalbumin control diet at a rate of 0.29 g nitrogen/day. Rats gaining weight at a rate of at least 3 g/day were randomly assigned to either a TPN diet or the control diet.

For those animals assigned to a TPN diet, catheters were inserted, saline was infused, they were allowed the post-surgery recovery and given four days of the -Arg TPN infusion, as described previously. One of four levels of +Arg diet was then infused over the next five days. The four calculated levels of arginine supplementation were 1.6, 2.5, 3.2 and 4.8%. The lactalbumin fed control animals were

also housed in individual metabolic cages and remained on the supplemented lactalbumin diet at the rate of 0.29 g N/day.

Twenty-four-hour urine collections were made as described earlier on both control and operated animals beginning on the first day post-surgery. Urine analysis included orotate, urinary urea, creatinine, citrate and total nitrogen. Urinary orotate concentrations were determined at least every other day to verify the presence of orotic aciduria and to follow the return of orotic acid excretion to more normal levels.

The experiment was terminated by exsanguination of the animals via the inferior vena cava under anesthesia. Approximately 7-10 mL of venous blood were collected in heparinized syringes; the liver, kidneys, spleen, small bowel, heart and lungs were excised. Catheter placement and general status of catheter entry site were checked and observations were recorded. The mesentery was removed from the small bowel at the time of excision. Upon excision, organs were immediately placed in ice cold saline, with the exception of liver and kidney used for urea cycle enzyme activity determination. Once all organs had been excised they were blotted dry and weighed.

A one-way analysis of variance was performed on organ weights and urinary metabolites to determine differences between groups. An adequate supplementation level was

determined as that level of supplementation which decreased and maintained an orotic acid excretion of ≤ 100 $\mu\text{g/day}$ within 48 hours. This was determined from regression analysis of urinary orotate vs. % arg over the linear portion of the curve (Robbins et al., 1979).

Assessing Urea Cycle Enzyme Activities under TPN

After acclimatization to the laboratory, rats assigned to TPN-feeding were catheterized, allowed to recover and administered the -Arg diet. Arginine was supplemented at a level of 4.5% arg as determined from the results of the protocol to establish arginine adequacy. Arg-supplemented lactalbumin-fed animals served as controls and began the experiment at the time of catheterization of the TPN-fed animals. Liver CPS-I, AS and A and kidney AS and A activities were determined in one-half of the animals in both groups at the end of the arg-devoid period. The same enzyme activities were determined on the remaining animals in both groups at the end of the arg-supplemented period. Enzyme activities were determined within two hours of excision, as outlined previously.

Twenty-four-hour urine collections were made and urinary metabolites analyzed. Organs were excised and weighed at the termination of the experiment. A one-way analysis of variance was performed on organ weights and

enzyme activities to determine differences between arg-devoid and arg-supplementation feeding.

Determination of Arteriovenous Amino Acid Differences

Animals were adapted, and those assigned to TPN-feeding were catheterized, allowed to recover and administered -Arg diet. Arg was supplemented at the level of 4.5% arg as determined from the results obtained from the protocol to establish arg adequacy. Supplemented lactalbumin-fed animals served as controls and began the experimental period at the time of catheterization for the TPN-fed animals. Blood flow rates and plasma amino acid concentrations in the hepatic, portal and left renal veins and the carotid artery were determined at the end of the arg-devoid period in one-half of the animals of both groups and at the end of the arg-supplemented period in the remaining animals. Blood flow rates were determined according to the method established by Welbourne et al. (1986). Animals were anesthetized and a tracheotomy was performed. TPN rats were continued on diet infusion after being anesthetized. The carotid artery was catheterized and 0.1 mL of 1000 USP units/mL heparin (Elkins-Sinn, Inc. Cherry Hill, NJ) was infused. Needle catheters (25 gauge) were inserted into the portal, hepatic and left renal veins. A 30 gauge needle catheter was inserted into one of the

tertiary branches of the mesenteric vein. An infusion of ^{14}C labeled para-aminohippurate (^{14}C -PAH) was initiated and continued 45 min to allow time for the label to reach steady state concentrations at a rate of 0.039 mL/min. At the end of the infusion period, approximately 0.8 mL of blood was slowly withdrawn from each vessel in the following order: hepatic, portal, renal and carotid. An aliquot of 0.3 mL whole blood was precipitated with 0.3 mL of 10% perchloric acid and centrifuged. An aliquot of 500 μL of the supernatant was placed into a scintillation vial and 20 mL ScintiVerseTM II were added. The samples were counted in a Beckman LS2800 scintillation counter (Beckman Instruments, Inc., Fullerton, CA) for 10 minutes at channel settings corresponding to ^{14}C pulses. An aliquot of the infusate was also prepared for counting in the same manner as the blood. Flow rate was calculated as follows: $(\text{cpm infusate} / (\text{cpm vein} - \text{cpm artery}) \times \text{infusate flow rate} \times (1/\text{BW}/100\text{g}))$. The remaining whole blood was prepared for amino acid analysis as described previously.

RESULTS AND DISCUSSION

This section will be divided according to the objectives for this dissertation research. The results of each objective will be discussed individually followed by a summary discussing the overall results of the dissertation research.

Development of a TPN Model for Determining Arginine Adequacy

Four experiments were performed to accomplish this objective. The first experiment was a preliminary one which established the effects of either a +Arg of 4.7% arg or a -Arg TPN diet on rats. Since this was a preliminary experiment, animals not needed for other experiments conducted in our laboratory were used even though the initial body weights were higher than those expected to be used for future experiments. A total of eight rats were catheterized and TPN administration began immediately. Six rats completed the four-day experiment; two animals became infiltrated with diet and had to be removed from the experiment. Table 3 lists the body and organ weights at the termination of this experiment. Final body weight was

Table 3. Body and Organ Weights after 96 Hours of 4.7% Arginine or 0% Arginine TPN.

	4.7% Arg (Ave $\pm$ SD)	0% Arg (Ave $\pm$ SD)
Final Body Weight (g)		
	379.67 $\pm$ 15.58	358.67 $\pm$ 20.07
Weight Gain (g/d)		
	-0.50 $\pm$ 0.54	0.83 $\pm$ 1.50
Organ Weight (g/100g BW)		
Liver	5.08 $\pm$ 0.24	3.88 $\pm$ 0.32
Kidney	0.91 $\pm$ 0.05	0.84 $\pm$ 0.07
Spleen	0.53 $\pm$ 0.20	0.37 $\pm$ 0.08
Lung	0.73 $\pm$ 0.13	0.62 $\pm$ 0.09
Heart	0.36 $\pm$ 0.04	0.35 $\pm$ 0.03

greater in the +Arg group due to a heavier initial weight. Despite the heavier final weight, the +Arg group lost an average of 0.5 g per day whereas the -Arg group gained an average of 0.83 g per day. This difference in weight gain may have been due to individual animal response to surgical recovery and adaptation to TPN which was confounded by the short duration of the experiment. Statistical differences were not determined due to the short duration of the experiment and movement of the rats to balance the groups on Day 2. Except for heart weight, all organs were heavier in the +Arg group relative to the -Arg group. Liver and spleen exhibited the greatest differences of 23.6 and 30.2%, respectively. Figure 2 shows the daily urinary orotate excretion for both groups in this experiment. Orotate excretion nearly doubled in the -Arg group within 24 hrs of TPN with a peak orotate excretion of approximately 1400 $\mu\text{g/day}$ on Day 3, relative to the +Arg group. Urinary citrate excretion shows a steady increase in citrate excretion in the -Arg group relative to the +Arg group throughout the duration of the experiment (Figure 3). Peak citric acid excretion in the -Arg group occurred on Day 4 and was four-fold greater than the +Arg group. Figure 4 showed the urinary urea nitrogen (UUN) excretion data for this experiment. There was little difference in UUN excretion between the two groups during the first two days

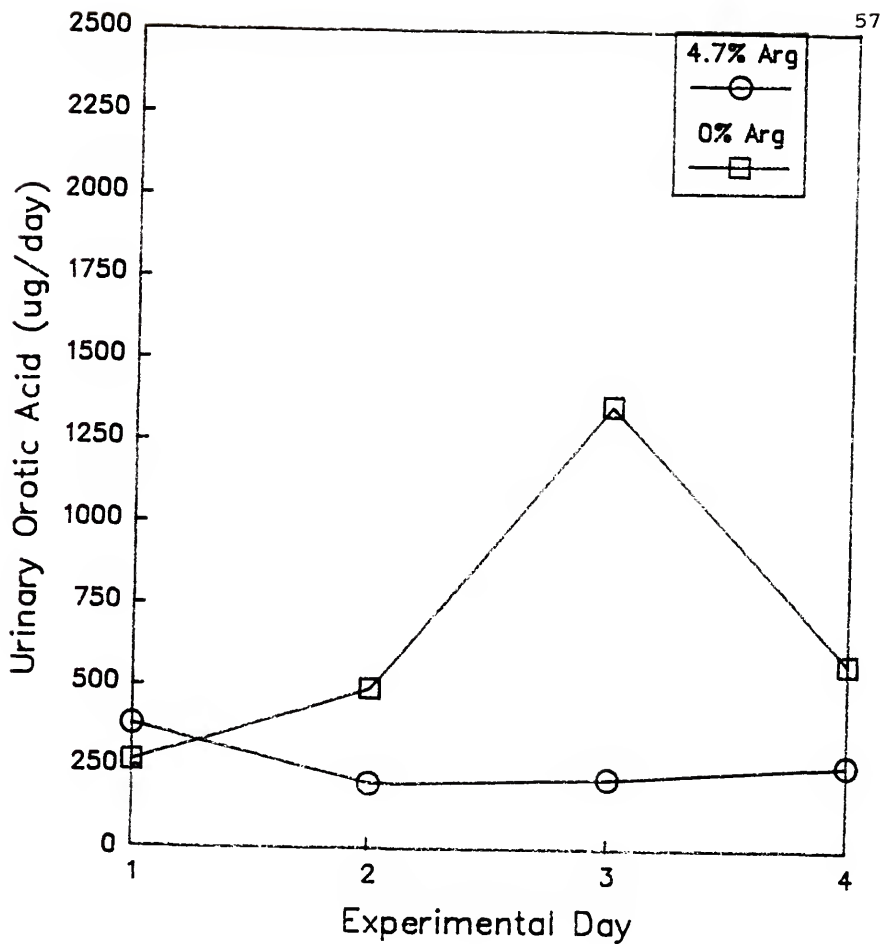


Figure 2. Average Daily Urinary Orotic Acid Excretion of Rats Administered a TPN Formula Supplemented with Either 4.7% Arginine or 0% Arginine per Total Amino Acids for Four Days.

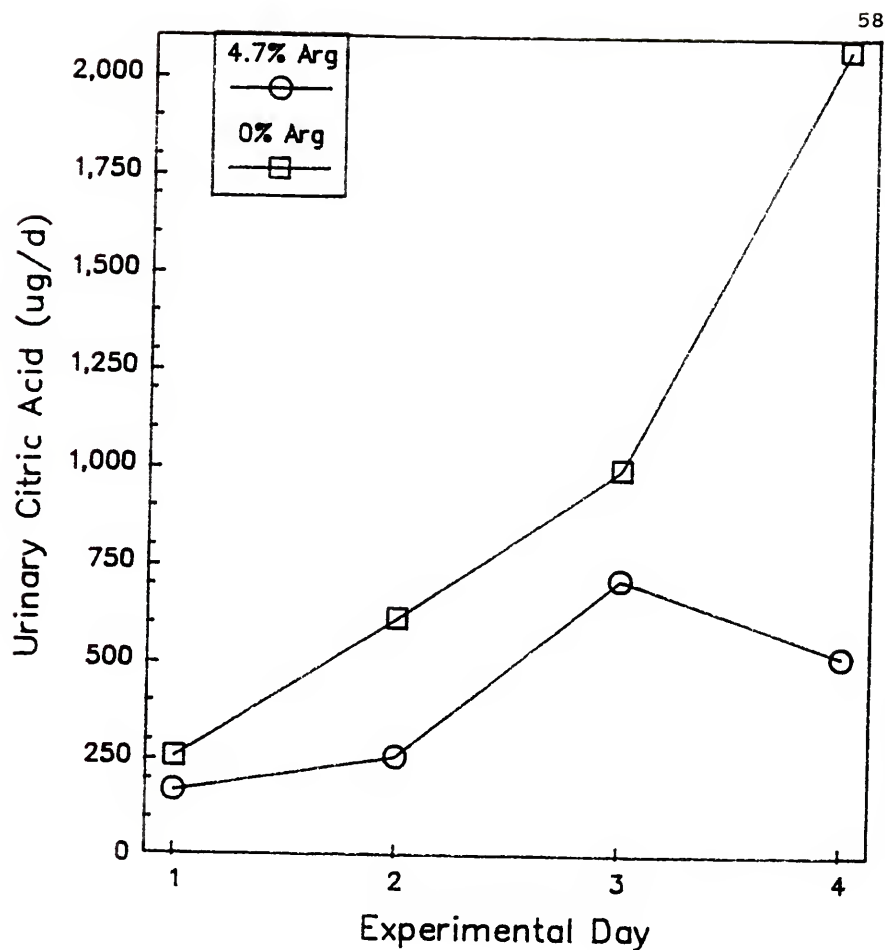


Figure 3. Average Daily Urinary Citric Acid Excretion of Rats Administered a TPN Formula Supplemented with Either 4.7% Arginine or 0% Arginine per Total Amino Acids for Four Days.

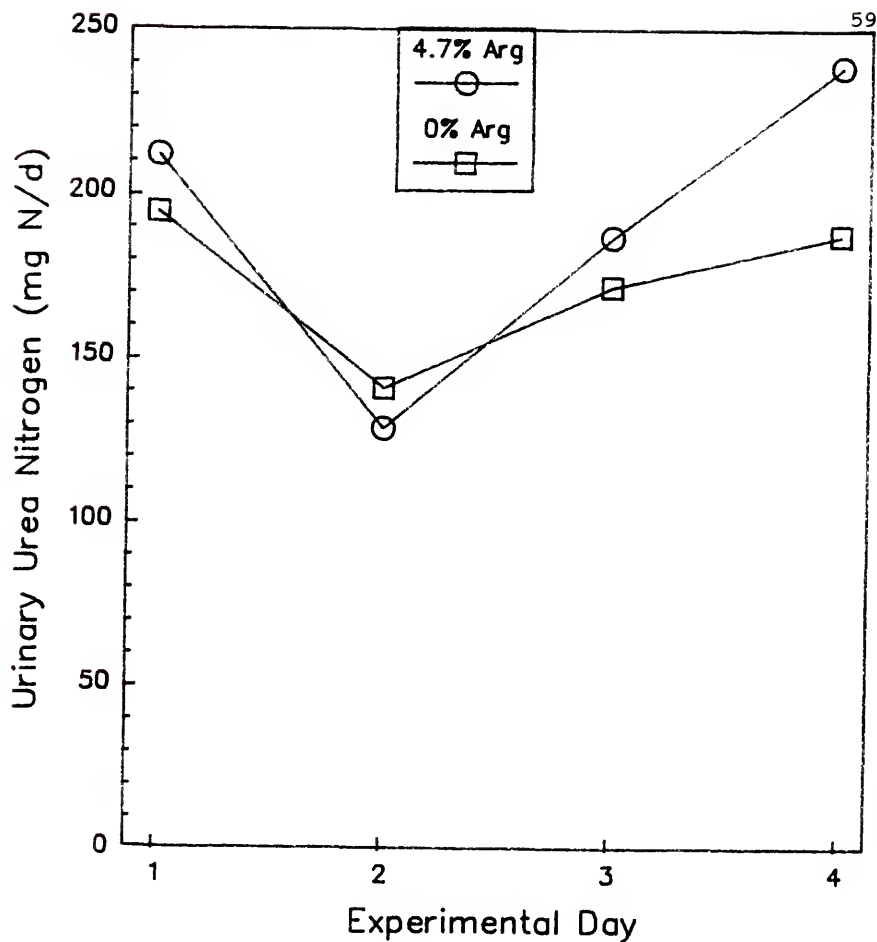


Figure 4. Average Daily Urinary Urea Nitrogen Excretion of Rats Administered a TPN Formula Supplemented with Either 4.7% Arginine or 0% Arginine per Total Amino Acids for Four Days.

of infusion. The +Arg group showed a slight, but steady increase in UUN from days three to four, relative to the -Arg group. Total daily urine volume is depicted in Figure 5. The +Arg group excreted slightly more urine on all days except the first, relative to the -Arg group. Urinary creatinine (Figure 6) was slightly elevated in the +Arg group relative to the -Arg group throughout the entire experiment. The coefficient of variation (CV) for creatinine excretion of individual rats varied between 3.7 and 9.9% with one animal having a CV of 18.3%. Data from this experiment for urinary orotate and citrate concur with data presented by Milner and Visek (1975) for characteristic urinary metabolite response in rats due to an arginine-deficient diet. Urinary creatinine and total urine volume followed the same pattern as data presented by Milner et al. (1974). Both of these parameters were depressed in the -Arg group. In humans, there is a normal variation in daily creatinine excretion of 4 to 8%; stress (emotional) and trauma can increase this variation by 5 to 10% and 20 to 100%, respectively (Heymsfield et al. 1983). Fisher (1965) reported urinary creatinine CV between 10 and 20% for rats fed a 15% protein stock diet. Variations of urinary creatinine within normal limits have been used as a verification for completeness of urine collection. The creatinine CV of individual rats from this experiment indicated that complete urine collections were obtained.

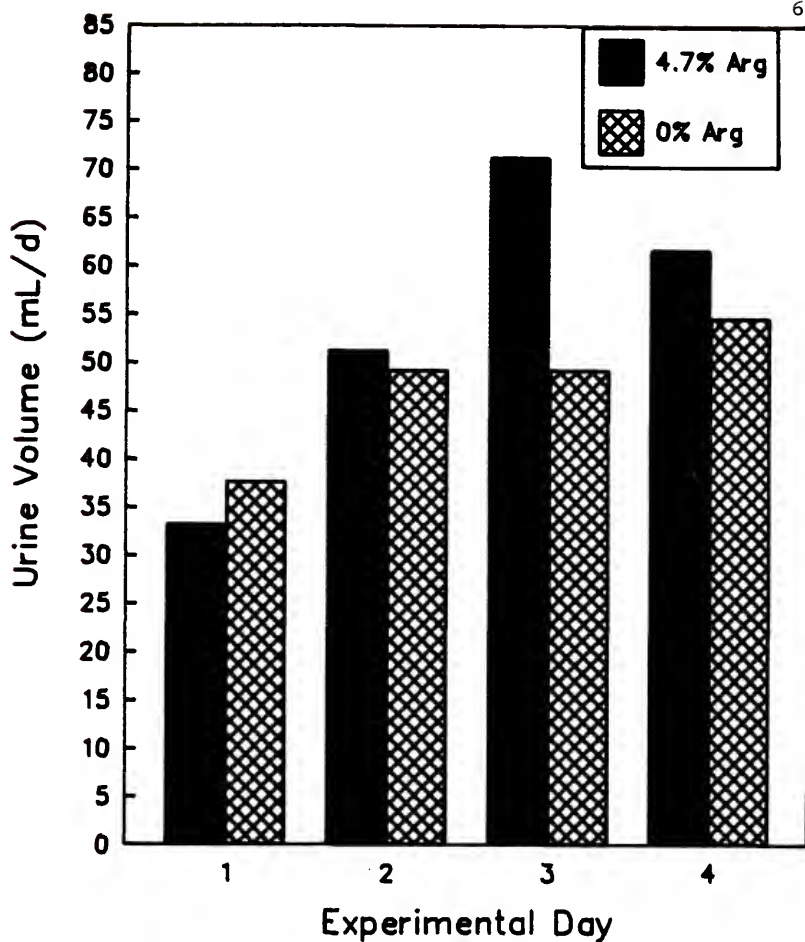


Figure 5. Average Daily Urine Volume of Rats Administered a TPN Formula Supplemented with Either 4.7% Arginine or 0% Arginine per Total Amino Acids for Four Days.

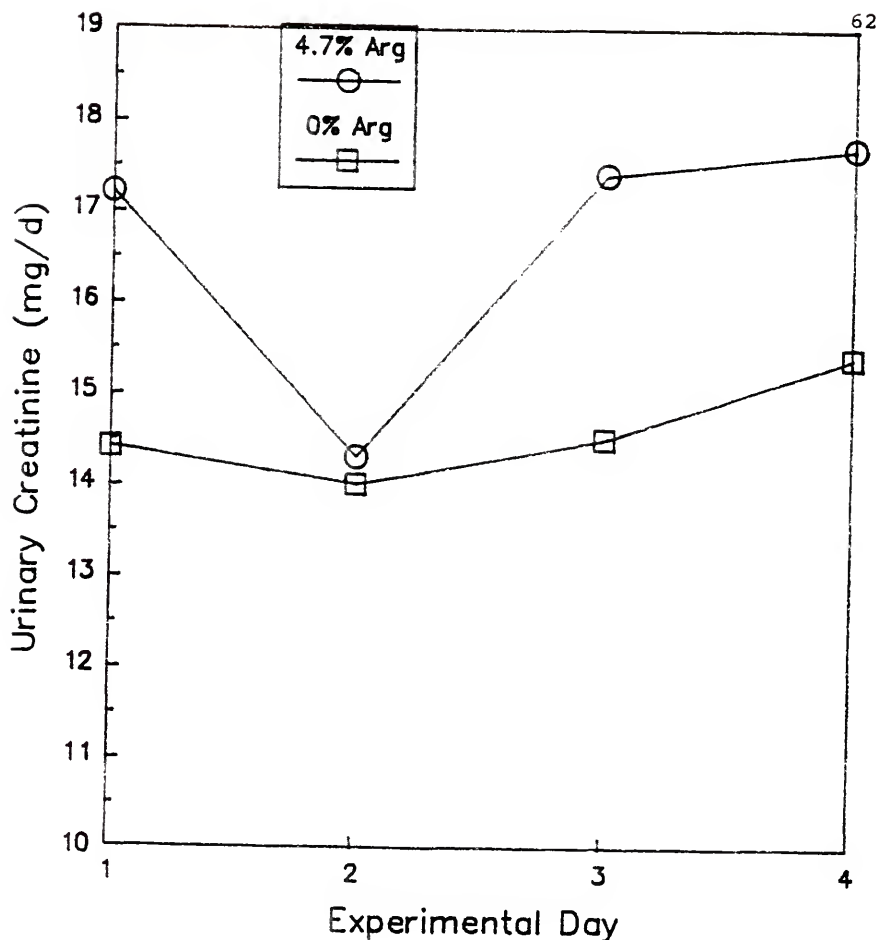


Figure 6. Average Daily Urinary Creatinine Excretion of Rats Administered a TPN Formula Supplemented with Either 4.7% Arginine or 0% Arginine per Total Amino Acids for Four Days.

Only urinary urea excretion in this experiment contrasted with data presented by Milner and Visek (1975).

Urea cycle enzyme activities measured in this experiment are listed in Table 4. Liver urea cycle enzymes were all elevated after 96 hours of -Arg TPN infusion relative to the +Arg group. Liver enzyme activities in the -Arg group were 194%, 148% and 141% greater than the activities in the +Arg group for CPS, AS and A, respectively. Ha and Milner (1979) measured liver urea cycle activities in rats fed orally an arginine-deficient diet and reported a 164% increase in CPS activity relative to the control group. However, AS and A activities were not significantly increased by the arg-deficient diet relative to controls.

Kidney urea cycle enzyme activities were depressed in the -Arg group relative to the +Arg group. Kaysen and Strecker (1973) reported that arginase activity in the liver was approximately 30 times greater than the activity in the kidney. The liver:kidney arginase activity ratio in this experiment was 14.1 for the +Arg group and 29.6 for the -Arg group. The renal medulla and cortex were separated and arginase activities were measured in the cortical region. Snellman et al. (1979) compared renal arginase activities in rats fed either a 6% or 21% protein diet. They reported a renal arginase activity increase of 327% and 50% in the cortical region and the outer medullary region,

Table 4. Urea Cycle Enzyme Activities After 96 Hours of 4.7% Arginine or 0% Arginine TPN

Enzyme	4.7% Arg (mean $\pm$ SEM)	0% Arg (mean $\pm$ SEM)
Liver Enzyme Activity*		
CPS	$1.52 \times 10^7 \pm 0.3 \times 10^7$	$2.95 \times 10^7 \pm 0.7 \times 10^7$
AS	33.98 ± 3.5	50.13 ± 6.4
A	$6.06 \times 10^4 \pm 0.7 \times 10^4$	$8.55 \times 10^4 \pm 1.7 \times 10^4$
Kidney Enzyme Activity*		
AS	34.78 ± 7.7	26.28 ± 3.4
A	$4.30 \times 10^3 \pm 0.7 \times 10^3$	$2.89 \times 10^3 \pm 1.7 \times 10^3$

* $\mu\text{mol product/hr/g tissue}$

CPS = carbamoyl phosphate synthetase

AS = argininosuccinate synthetase

A = arginase

respectively, in rats on the low protein diet. Total dietary protein was equivalent in this experiment; thus, the increased kidney urea cycle enzyme activities in the +Arg group may be due to increased substrate supply.

The conclusion of this experiment was that TPN-administered rats have a similar response to an arg-deficient diet as rats orally-fed an arg-deficient diet. Thus, this animal protocol provides an adequate model to study arginine metabolism in parenteral nutrition.

The second experiment was designed to determine the effect of route of administration as well as alternate substrate supply on arginine metabolism when compared to TPN-administered experiments. Thus, the TPN diet was orally-fed. The experiment utilized three groups of seven animals each. The first group received an orally-fed TPN diet supplemented first to 0.78% arg and then 1.61% gln, each after a three-day -Arg period. The second group received an orally-fed TPN diet supplemented first to 3.23% arg and then 5.36% gln, again, after a three-day -Arg period. The third group served as a control group and was pair-fed isonitrogenously an unsupplemented lactalbumin diet.

Figure 7 shows the growth curve of the three groups over the course of the experimental period. Table 5 shows the average body weights of the three groups initially as well as at the transition between supplementation levels of

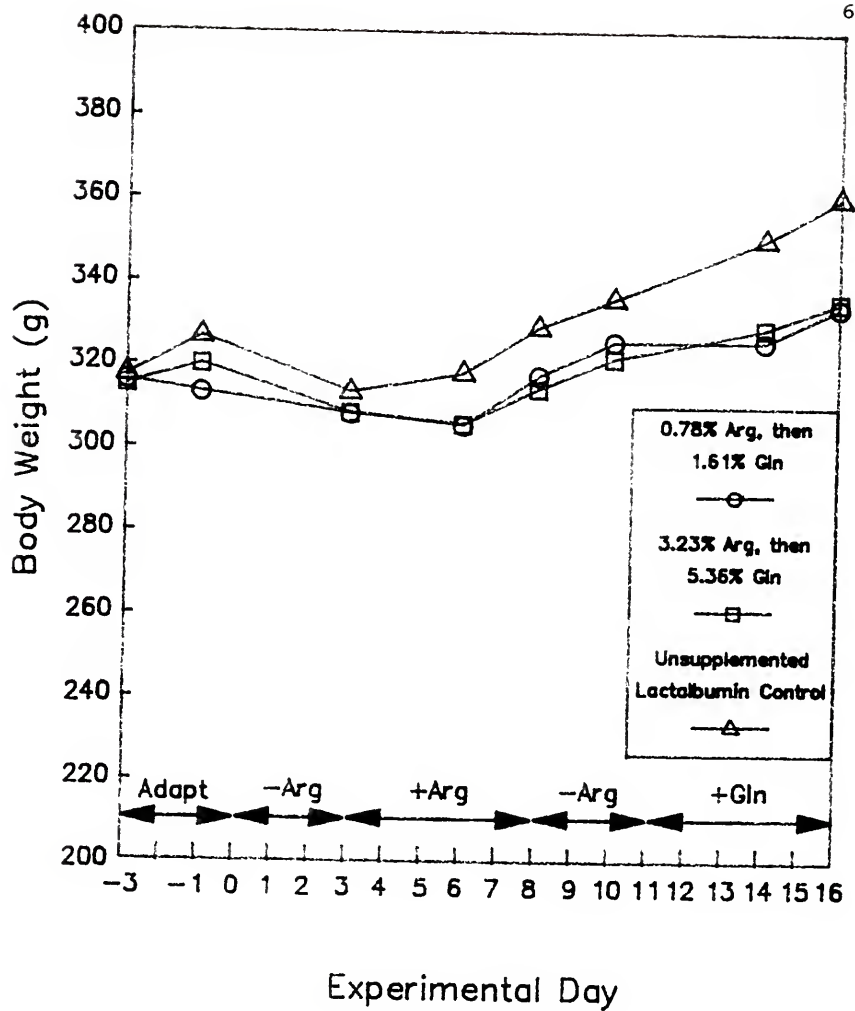


Figure 7. Average Body Weight Gain of Rats Orally-Fed One of Two Levels of Arginine, then, One of Two Levels of Glutamine Supplemented TPN Formula and Controls.

Table 5. Body Weights at Selected Intervals for Rats Orally-Fed a TPN Diet.

Time Period	Low Level	High Level (g, mean $\pm$ SD)	Control
Initial	270.00 $\pm$ 6.0 ^a	270.71 $\pm$ 18.3 ^a	263.29 $\pm$ 11.5 ^a
Adaptation	313.14 $\pm$ 20.7 ^a	319.86 $\pm$ 29.4 ^a	326.57 $\pm$ 13.0 ^a
First -Arg	308.14 $\pm$ 12.5 ^a	308.29 $\pm$ 22.0 ^a	313.29 $\pm$ 10.1 ^a
+Arg	317.43 $\pm$ 12.7 ^a	314.28 $\pm$ 20.8 ^a	329.14 $\pm$ 8.2 ^a
Second -Arg	325.57 $\pm$ 13.6 ^a	321.57 $\pm$ 26.2 ^a	335.86 $\pm$ 97. ^a
+Gln	334.14 $\pm$ 12.1 ^b	335.42 $\pm$ 23.8 ^b	360.43 $\pm$ 10.5 ^a

*Except initial body weight, all weights given are those at the end of the time period specified. Initial refers to arrival to the laboratory, adaptation refers to the end of the adaptation period, first and second -Arg refer to the end of the -Arg periods, +Arg refers to 0.78% arg for the low level and 3.23% arg for the High Level and +Gln refers to 1.61% gln for the Low Level and 5.38% for the High Level.

Values without common superscript within a row differ by $p < 0.05$.

the diets. There were no statistical differences in average body weight between the three groups upon arrival at the laboratory, at the end of the ad-libitum-fed adaptation period or during the first -Arg and +Arg phase of the experiment. However, there was a trend toward a decreased body weight in both orally-fed TPN diet groups relative to the control group. By the end of the +Gln period, there was a difference ($p \leq 0.05$) between the control animals and the two orally-fed TPN diet groups, but no difference in body weight between the two orally-fed TPN diet groups. All three groups lost weight during the first -Arg period due to low food consumption; but did not lose weight during the second -Arg period (Table 5 and Table 6). During the first -Arg period, food consumption (expressed as N intake) in the control animals was statistically less relative to the two orally-fed TPN diet groups (Table 6). The difference in food consumption was concluded to be due to a lack of adaptation to the diet which resulted in an initial refusal of the semipurified diet. This refusal lead to the body weight loss during the initial -Arg period. Except on Days 12 and 13, there was no difference ($p \leq 0.05$) in food consumption between the two orally-fed TPN diet groups. The weight loss during the first -Arg period for these two groups was concluded to be due to a lack of adaptation to an isonitrogenous diet as well as a response to the -Arg diet.

Table 6. Food Consumption as Expressed by Nitrogen Intake for Orally-Fed TPN Diet Rats.

Day	Low Level	High Level (mg N, mean $\pm$ SD)	Control
1	230.57 $\pm$ 45.8 ^a	252.00 $\pm$ 25.3 ^a	150.14 $\pm$ 56.3 ^b
2	273.43 $\pm$ 27.8 ^{a,b}	281.14 $\pm$ 8.5 ^a	259.57 $\pm$ 12.6 ^b
3	276.14 $\pm$ 8.6 ^{a,b}	282.14 $\pm$ 6.7 ^a	266.71 $\pm$ 13.8 ^b
4	283.86 $\pm$ 5.0 ^{a,b}	287.14 $\pm$ 2.7 ^a	280.57 $\pm$ 3.6 ^b
5	285.57 $\pm$ 3.7 ^a	281.57 $\pm$ 3.2 ^a	283.56 $\pm$ 5.0 ^a
6	285.71 $\pm$ 1.9 ^a	284.00 $\pm$ 3.9 ^{a,b}	277.86 $\pm$ 8.8 ^b
7	286.43 $\pm$ 2.4 ^a	280.29 $\pm$ 8.8 ^a	277.86 $\pm$ 8.8 ^a
8	266.14 $\pm$ 29.9 ^a	267.71 $\pm$ 29.3 ^a	268.43 $\pm$ 9.7 ^a
9	248.29 $\pm$ 31.1 ^a	262.00 $\pm$ 32.2 ^a	244.57 $\pm$ 6.4 ^a
10	249.86 $\pm$ 35.3 ^a	243.57 $\pm$ 71.0 ^a	244.57 $\pm$ 1.1 ^a
11	252.43 $\pm$ 43.1 ^a	274.71 $\pm$ 9.8 ^a	251.57 $\pm$ 9.3 ^a
12	237.57 $\pm$ 35.1 ^b	268.29 $\pm$ 30.6 ^a	286.57 $\pm$ 2.0 ^a
13	265.86 $\pm$ 12.0 ^b	283.86 $\pm$ 3.4 ^a	276.43 $\pm$ 9.1 ^a
14	279.00 $\pm$ 3.4 ^a	267.14 $\pm$ 22.1 ^a	257.00 $\pm$ 26.8 ^a
15	260.71 $\pm$ 31.1 ^a	273.14 $\pm$ 21.9 ^a	241.14 $\pm$ 38.3 ^a
16	247.43 $\pm$ 39.5 ^a	268.43 $\pm$ 23.6 ^a	272.29 $\pm$ 13.3 ^a

Days 1-3 were first -Arg diet; Days 4-8 were +Arg at 0.78% arg in Low Level and 3.23% arg in High Level; Days 9-11 were second -Arg diet; Days 12-16 were +Gln at 1.61% gln in Low Level and 5.38% gln in High Level. Control was fed unsupplemented lactalbumin control diet.

Values without common superscript within a row differ by $p < 0.05$.

Table 7 lists the organ weights at the termination of the experiment. There were no differences ($p \leq 0.05$) in organ weights, except spleen weight, due to diet. Spleen weight was lower ($p \leq 0.05$) in both orally-fed TPN groups relative to the control group.

Feeding the rats a TPN diet orally increased the urine output (Figure 8). Only Day 1 differed from the pattern of no statistical difference between the two orally-fed TPN diet groups and these two groups differed from the control group. On Day 1, the two orally-fed TPN diet groups excreted more urine than controls, however, all three groups were different ($p \leq 0.05$) from each other.

Figure 9 shows the daily urinary orotate excretion levels. All groups exhibited orotic aciduria throughout the entire experiment, although this was not realized until further experiments had been conducted. Overall, there was not a clear trend in dietary influence on orotate excretion. Standard deviations within a group were very large and the coefficient of variation (CV) ranged from 12 to 148%. It was learned from subsequent experiments the urinary orotate excretion of rats, within a dietary group, not receiving adequate arginine had extremely large variations. During the first -Arg period, the orally-fed TPN diet groups tended toward higher orotate excretion by the second and third days of -Arg feeding, as expected. As a result of the large CV, there were no differences ($p \leq 0.05$) between any of the

Table 7. Organ Weights for Rats Orally-Fed a TPN Diet Containing Either 1.61% or 5.36% Glutamine and Control.

Organ	1.35% Gln (mean $\pm$ SD, g/100 g BW)	5.38% Gln (mean $\pm$ SD, g/100 g BW)	Control
Liver	3.57 $\pm$ 0.2 ^a	3.79 $\pm$ 0.2 ^a	3.54 $\pm$ 0.2 ^a
Kidney	0.58 $\pm$ 0.05 ^a	0.59 $\pm$ 0.04 ^a	0.60 $\pm$ 0.04 ^a
Spleen	0.21 $\pm$ 0.02 ^b	0.22 $\pm$ 0.03 ^b	0.26 $\pm$ 0.04 ^a
Lung	0.47 $\pm$ 0.03 ^a	0.46 $\pm$ 0.05 ^a	0.46 $\pm$ 0.02 ^a
Heart	0.37 $\pm$ 0.02 ^a	0.37 $\pm$ 0.03 ^a	0.36 $\pm$ 0.02 ^a
GI*	2.59 $\pm$ 0.25 ^a	2.70 $\pm$ 0.32 ^a	2.51 $\pm$ 0.18 ^a

*Small intestine from stomach to cecum with mesentery intact.

Values without a common superscript within a row differ by $p < 0.05$.

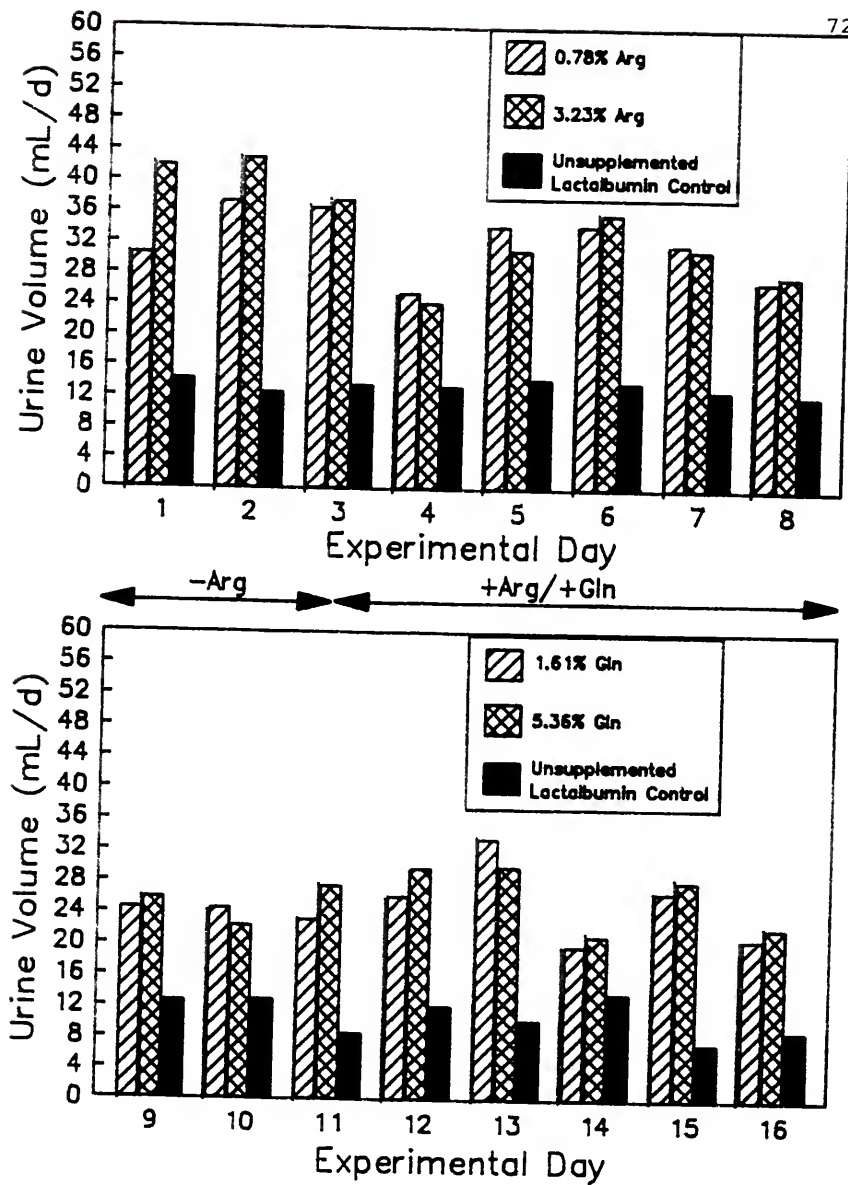


Figure 8. Average Daily Urine Volume of Rats Orally-Fed One of Two Levels of Arginine, then, One of Two Levels of Glutamine Supplemented TPN Formula and Control.

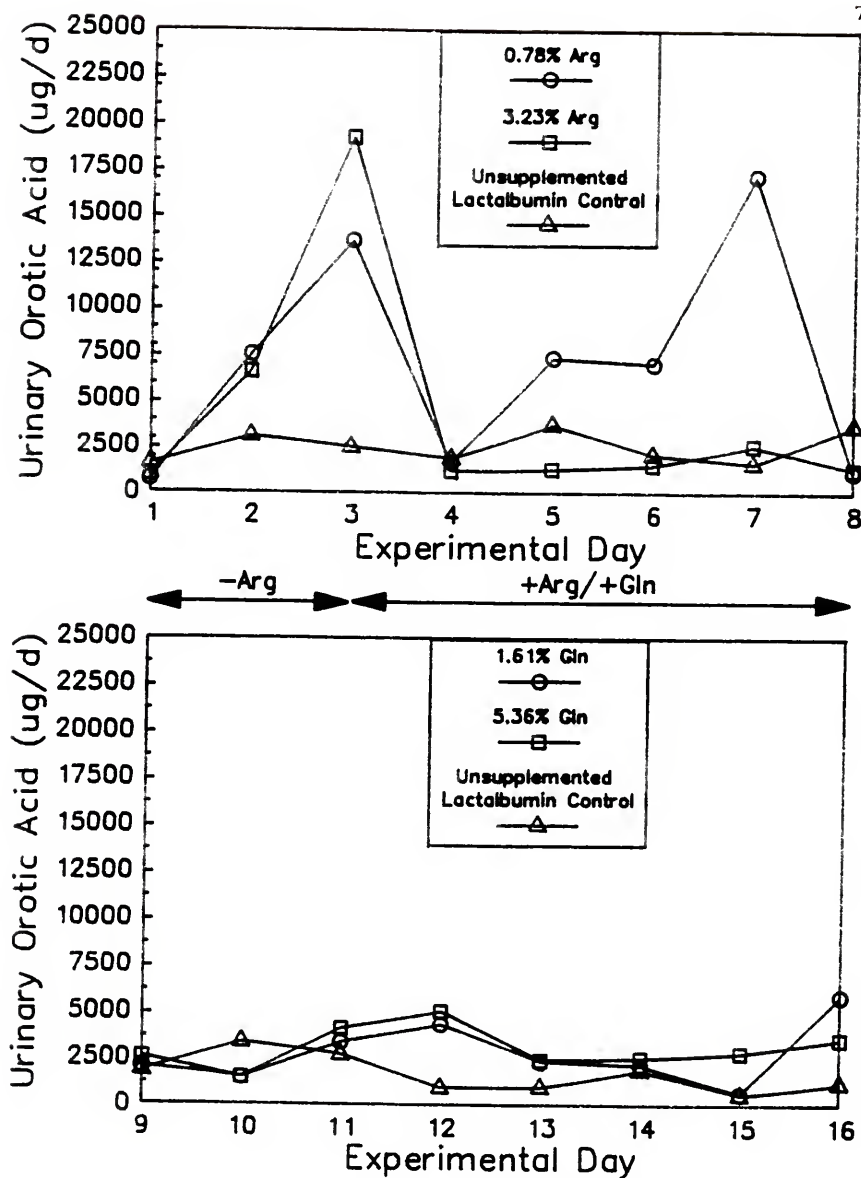


Figure 9. Average Daily Orotic Acid Excretion of Rats Orally-Fed One of Two Levels of Arginine, then, One of Two Levels of Glutamine Supplemented TPN Formula and Control.

groups on Day 2, although the orotate excretion was higher in the orally-fed TPN diet groups. On Days 1 and 3, all three groups were statistically different from each other. On Days 5 and 6, the 0.78% arg group excreted more orotate than the other two groups, however, only on Day 6 was this difference significant ($p \leq 0.05$). There were no differences in orotic acid excretion between any of the groups on Days 7 and 8. During the second -Arg period, the orally-fed TPN diet groups did not display the expected increase in orotic acid excretion. These two groups excreted less ($p \leq 0.05$) orotic acid at the end of the second -Arg period relative to the last day of the first -Arg period. After initiation of +Gln, the two orally-fed TPN diet groups generally excreted more orotic acid than the control animals. However, only on Days 12 and 16 were the excretion values different ($p \leq 0.05$) from controls. At this point in the research project, it was not known if rats fed a TPN diet were capable of excreting orotate at levels similar to those reported in the literature as 'control' levels for animals fed a purified diet. Thus, no target orotate excretion level had been chosen for an indication of adequate arginine supplementation. Figure 10 superimposes the urinary orotate data for the two phases of this experiment. The increase in orotic acid excretion during the first, but not the second -Arg period was concluded to

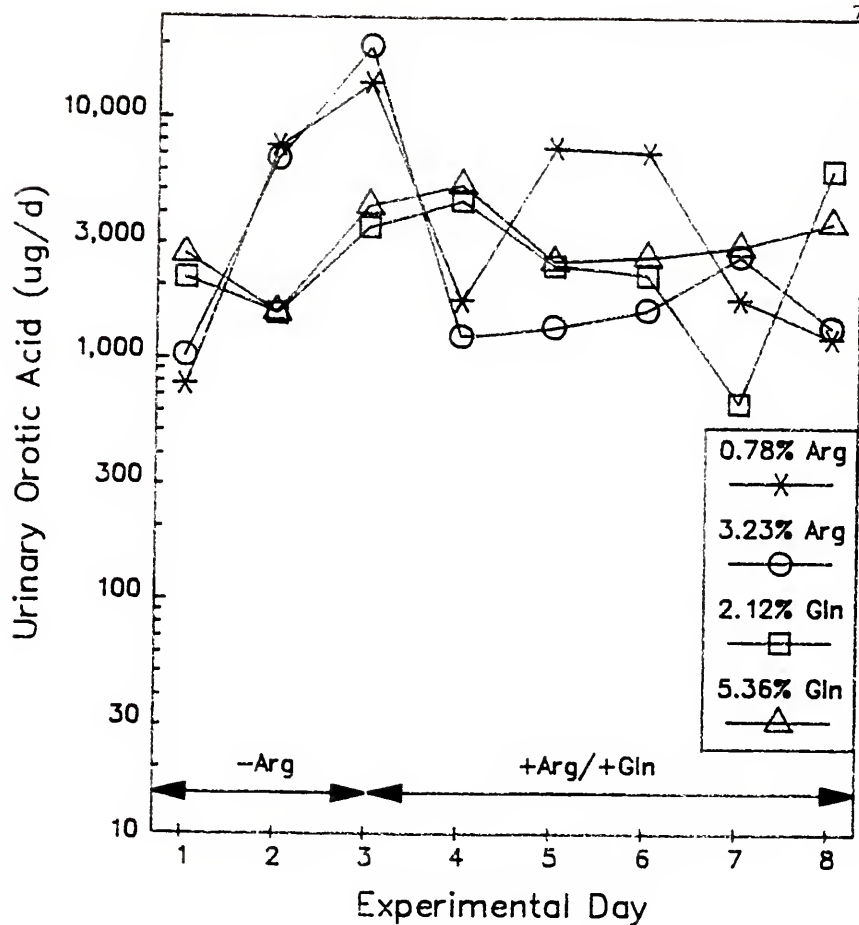


Figure 10. Superimposing Urinary Orotic Acid Excretion of Rats Orally-Fed One of Two Levels of Arginine, then, One of Two Levels of Glutamine Supplemented TPN Formula.

be partially due to feeding a 21% protein laboratory ration ad-libitum during the adaptation period.

Figure 11 depicts the daily urinary citrate excretion. Excretion levels are elevated in all groups on Day 1 and in the orally-fed TPN diet groups on Day 2, relative to the remainder of the experiment. However, after the second day of the experiment, citrate excretion was fairly uniform throughout the entire experiment within all groups. Citrate excretion in control rats was slightly elevated during the first five days of the experiment. There was no difference ($p \leq 0.05$) in excretion between the groups for Days 3 through 5. For the remaining +Arg period, the orally-fed TPN diet groups were not statistically different from each other, but were higher than controls. During the second phase of this experiment, citrate excretion was statistically higher in the orally-fed TPN groups relative to the control group, except Days 11, 14 and 16. There was a large variation in citrate excretion on Day 11 in both the orally-fed TPN diet groups and this variation probably led to lack of statistical difference between the three groups on this day. There was no difference in citrate excretion by a paired-t analysis of the data from the end of the various periods for either of the orally-fed TPN diet groups. However, there was a statistical difference in citrate excretion shown by the paired-t analysis for control animals between the two -Arg periods and between the +Arg

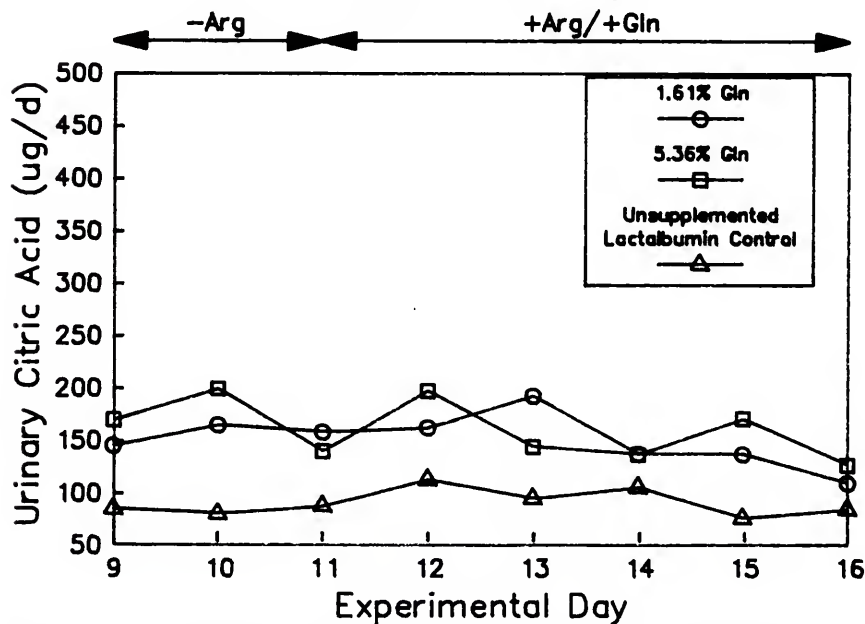
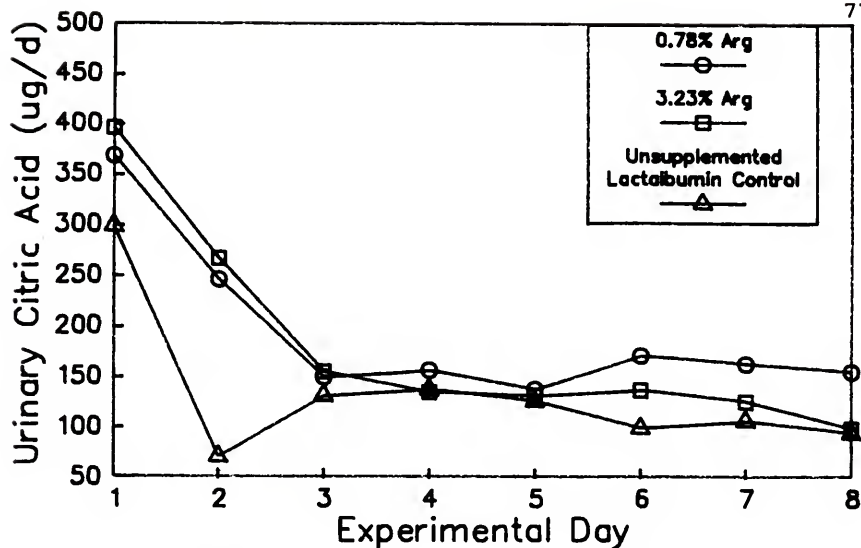


Figure 11. Average Daily Urinary Citric Acid Excretion of Rats Orally-Fed One of Two Levels of Arginine, then, One of Two Levels of Glutamine Supplemented TPN Formula and Control.

and the first -Arg period, but not for the +Gln and second -Arg period.

Urinary urea nitrogen (UUN) excretion for this experiment is shown in Figure 12. There was a steady decline in UUN for the first five days of the experiment for all groups, and thereafter, UUN excretion remained fairly constant. Control animals excreted a greater amount of UUN relative to the two orally-fed TPN diet groups. Except Days 1, 2 and 6, control animals excreted statistically higher amounts of UUN, relative to the two orally-fed TPN diet groups. There was no difference ($p \leq 0.05$) in UUN excretion between the two TPN diet groups, except all groups were statistically different from each other on Days 2 and 6. On the first day, the control and 3.23% arg group differed statistically ($p \leq 0.05$) in UUN excretion. There was a difference in paired-t analysis for all groups between the two -Arg periods and between the +Arg and first -Arg period. However, there was no difference in paired-t analysis for UUN between the +Gln and second -Arg period.

Urinary total nitrogen for this experiment is shown in Figure 13. Urinary total nitrogen followed the same pattern as UUN. There was a decline in total nitrogen excretion for the first five days and a relatively constant excretion level thereafter. Except for Day 2, control rats excreted more total nitrogen relative to the two orally-fed TPN groups. The difference was statistically greater after

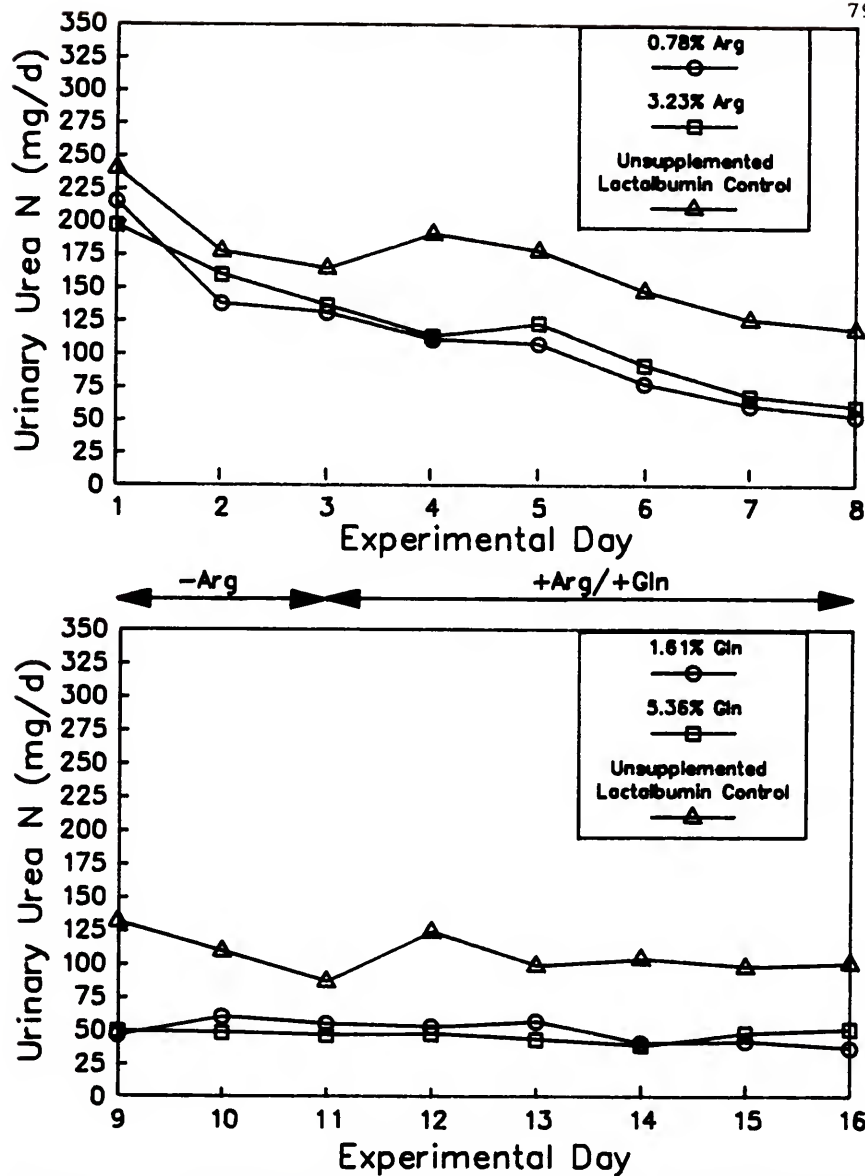


Figure 12. Average Daily Urinary Urea Nitrogen Excretion of Rats Orally-Fed One of Two Levels of Arginine, then, One of Two Levels of Glutamine Supplemented TPN Formula and Control.

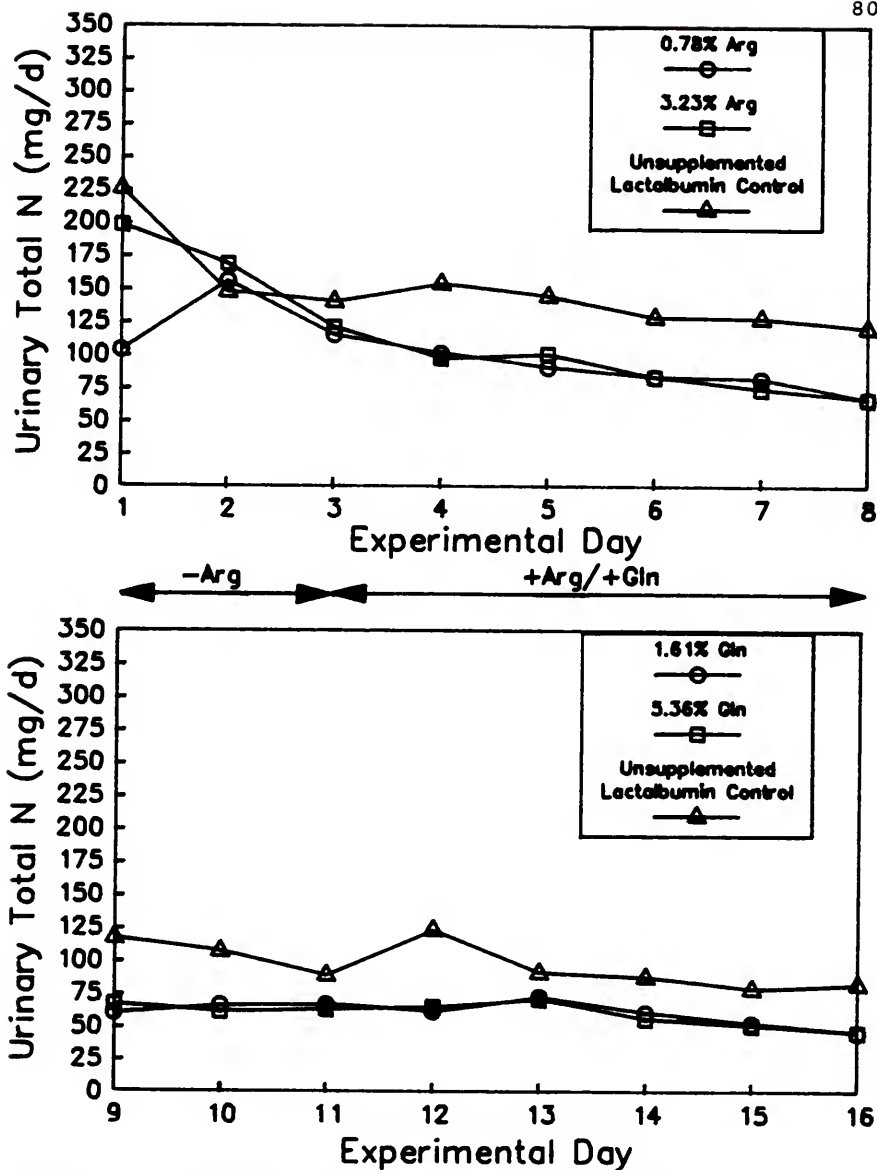


Figure 13. Average Daily Urinary Total Nitrogen Excretion of Rats Orally-Fed One of Two Levels of Arginine, then, One of Two Levels of Glutamine Supplemented TPN Formula and Control.

Day 2. Except Day 2, there was no statistical difference in urinary total nitrogen excretion between the two orally-fed TPN diet groups. There was a difference in urinary total nitrogen (paired-t analysis, $p \leq 0.05$) for the orally-fed TPN diet animals between the two -Arg periods, the +Arg and the first -Arg period and the +Gln and second -Arg period. There was a difference ($p \leq 0.05$) in urinary total nitrogen for the control animals during the two -Arg periods and the +Arg and first -Arg period. However, there was no statistical difference between the +Gln and second -Arg period for controls.

Daily urinary creatinine excretion is shown in Figure 14. Urinary creatinine excretion is fairly uniform throughout the entire experimental period. Except on the last day of the first phase of the experiment (Day 8), there was no statistical difference in urinary creatinine excretion between the three groups. On Day 8, the control group excreted a greater amount ($p \leq 0.05$) of creatinine relative to the two orally-fed TPN diet groups. The second phase of the experiment showed some statistical differences in creatinine excretion, however, the trend of significance was not uniform. On Days 12, 13, 14 and 16, the two orally-fed TPN diet groups were not statistically different from each other and except for Day 14, they were different from the control group. On Days 12, 14 and 16 the creatinine excretion level of these two groups was less than that of

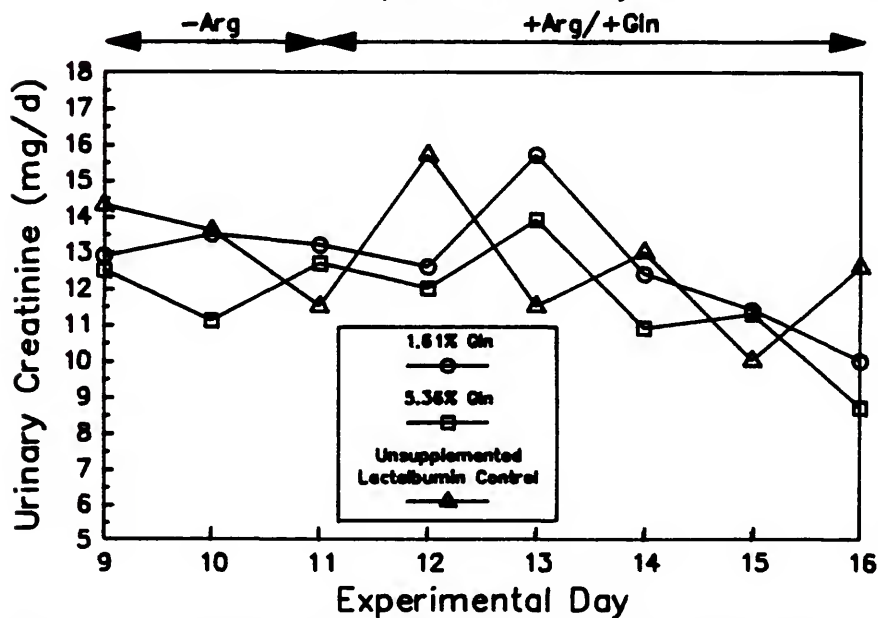
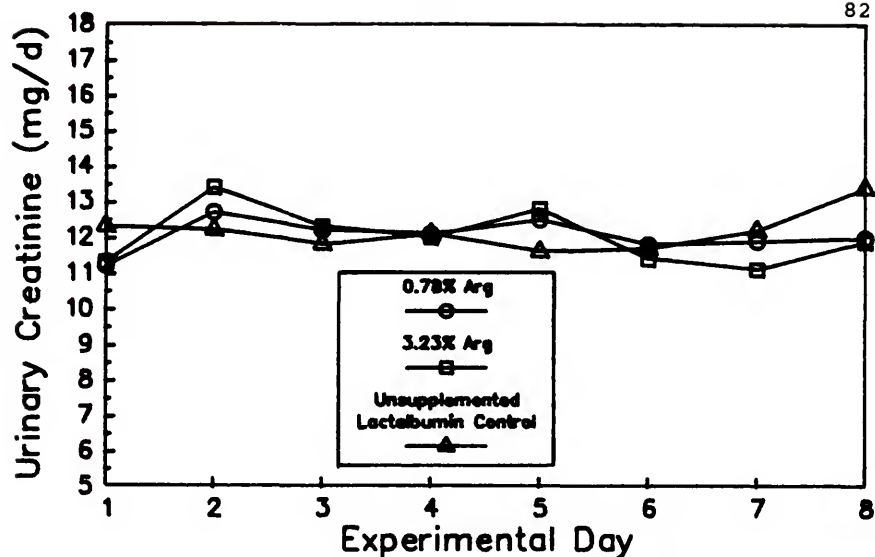


Figure 14. Average Daily Urinary Creatinine Excretion of Rats Orally-Fed One of Two Levels of Arginine, then, One of Two Levels of Glutamine Supplemented TPN Formula and Control.

the controls. However, on Day 13 these two groups excreted more creatinine than controls. The absolute difference in excretion was never more than 4.2 mg/d, thus there may not be a significant physiological difference in the creatinine excretion levels between any of these three diets.

Individual animal creatinine excretion CV was between 9.5 and 13.7 mg/d. There was no difference in creatinine coefficient of variation between any of the animals, except the one with the lowest variation in excretion level. Thus, it can be concluded that urine samples were adequately collected.

Urea cycle enzyme activities were measured at the termination of the experiment and activities are listed in Table 8. Liver AS activity was elevated, but not significantly ($p \leq 0.05$), in the orally-fed TPN diet groups relative to the control group, and the 1.61% gln group was elevated relative to the 5.36% gln group. Liver A activity was significantly depressed ($p \leq 0.05$) in the orally-fed TPN diet groups relative to the control group. The 1.61% gln group was depressed to a greater extent than the 5.36% gln group, but not significantly ($p \leq 0.05$). Kidney AS activity was depressed ($p \leq 0.05$) in both the orally-fed TPN groups relative to the control groups. There was no statistical difference in kidney A activity among any of the diets.

Plasma amino acids were measured at the termination of the experiment and are listed in Table 9. Serine, glycine

Table 8. Urea Cycle Enzyme Activities for Rats Orally-Fed a TPN Diet Containing Either 1.61% or 5.36% Glutamine and Control.

Enzyme	1.61% Gln	5.36% Gln (mean $\pm$ SD)	Control
Liver Enzyme Activity*			
CPS ($\times 10^6$)	3.38 $\pm$ 2.0	nd	nd
AS	56.75 $\pm$ 15.8 ^a	43.81 $\pm$ 12.5 ^a	46.72 $\pm$ 27.2 ^a
A ($\times 10^5$)	6.24 $\pm$ 4.2 ^b	9.74 $\pm$ 1.1 ^b	14.00 $\pm$ 4.5 ^a
Kidney Enzyme Activity*			
AS	10.83 $\pm$ 5.2 ^b	17.15 $\pm$ 3.3 ^b	53.32 $\pm$ 21.4 ^a
A ($\times 10^3$)	8.68 $\pm$ 9.4 ^a	2.50 $\pm$ 0.6 ^a	3.94 $\pm$ 1.3 ^a

* $\mu\text{mol product/hr/g tissue}$

CPS = carbamoyl phosphate synthetase

AS = argininosuccinate synthetase

A = arginase

Values without a common superscript within a row differ by $p < 0.05$.

Table 9. Plasma Amino Acid Concentrations for Rats Orally-Fed a TPN Diet Containing Either 1.61% or 5.36% Glutamine and Control.

Amino Acid	1.61% Gln	5.36% Gln	Control
Aspartate	51 $\pm$ 8 ^c	61 $\pm$ 6 ^b	69 $\pm$ 5 ^a
Threonine	359 $\pm$ 26 ^b	370 $\pm$ 48 ^b	728 $\pm$ 50 ^a
Serine	1468 $\pm$ 132 ^a	1401 $\pm$ 94 ^a	578 $\pm$ 31 ^b
Asparagine	87 $\pm$ 11 ^b	103 $\pm$ 39 ^b	160 $\pm$ 32 ^a
Glutamate	438 $\pm$ 59 ^b	516 $\pm$ 69 ^a	418 $\pm$ 46 ^b
Glutamine	830 $\pm$ 103 ^a	600 $\pm$ 86 ^b	244 $\pm$ 75 ^c
Proline	354 $\pm$ 42 ^a	321 $\pm$ 46 ^a	333 $\pm$ 39 ^a
Glycine	3046 $\pm$ 433 ^a	2674 $\pm$ 483 ^a	532 $\pm$ 56 ^b
Alanine	1778 $\pm$ 122 ^a	1566 $\pm$ 380 ^a	907 $\pm$ 154 ^b
Citrulline	92 $\pm$ 15 ^a	108 $\pm$ 19 ^a	89 $\pm$ 16 ^a
Valine	174 $\pm$ 20 ^a	163 $\pm$ 26 ^a	168 $\pm$ 21 ^a
Cystine	18 $\pm$ 1 ^{a,b}	20 $\pm$ 3 ^a	16 $\pm$ 1 ^b
Methionine	81 $\pm$ 19 ^a	64 $\pm$ 13 ^b	69 $\pm$ 5 ^{a,b}
Isoleucine	119 $\pm$ 10 ^{a,b}	107 $\pm$ 16 ^b	130 $\pm$ 17 ^a
Leucine	134 $\pm$ 21 ^b	145 $\pm$ 16 ^b	242 $\pm$ 32 ^a
Tyrosine	56 $\pm$ 9 ^b	60 $\pm$ 12 ^b	78 $\pm$ 10 ^a
Phenylalanine	99 $\pm$ 8 ^a	82 $\pm$ 15 ^b	100 $\pm$ 10 ^a
Tryptophan	76 $\pm$ 24 ^b	82 $\pm$ 15 ^b	109 $\pm$ 16 ^a
Ornithine	201 $\pm$ 58 ^b	277 $\pm$ 44 ^a	243 $\pm$ 54 ^{a,b}
Lysine	222 $\pm$ 80 ^b	212 $\pm$ 47 ^b	681 $\pm$ 41 ^a
Histidine	40 $\pm$ 5 ^{a,b}	38 $\pm$ 10 ^b	47 $\pm$ 4 ^a
Arginine	88 $\pm$ 31 ^b	55 $\pm$ 50 ^b	136 $\pm$ 45 ^a

Values (mean $\pm$ SD, nmol/mL) without a common superscript within a row differ by $p \leq 0.05$.

and alanine concentrations are elevated ($p \leq 0.05$) in the orally-fed TPN diet groups relative to the control group. Threonine, asparagine, leucine, tyrosine, tryptophan, lysine and arginine concentrations are all depressed in the orally-fed TPN diet group relative to the control group. There was no difference in proline, valine and citrulline concentration due to diet. Aspartate and glutamine concentrations in each diet group were different. There was no distinct difference in glutamate, cystine, methionine, isoleucine, phenylalanine, ornithine or histidine concentrations due to diet. The amino acids which are most relevant to arginine metabolism and urea cycle enzyme efficiency are arginine, ornithine, citrulline, glutamine and lysine. Glutamine, which is thought to provide citrulline to the kidney to synthesize arginine for peripheral use was greatly elevated in the orally-fed TPN groups which was expected since the blood samples were taken during the glutamine supplementation period. It would be expected that the group receiving 5.36% gln would have had the highest concentration of venous glutamine; however, the group receiving 1.61% gln had the highest glutamine concentration. Citrulline was not different between groups, but arginine was lower in the orally-fed TPN diet groups. Lysine is an arginine antagonist due to competition for the y^+ carrier system in peripheral tissues. Lysine concentration was depressed in the orally-fed TPN groups relative to the control group. Thus, there should be a

minimal effect of lys on peripheral arg utilization. Ornithine was probably not physiologically different since Duncan's analysis produced overlapping in nonsignificance between the three diet groups.

The major conclusion of this experiment was that the animals must be adapted isonitrogenously to the subsequent TPN or control diet to avoid the continual decrease in nitrogen loss during the initial phase of the experiment. The data from this experiment indicated that by Day 5 of experimental diets, there was a plateau and thereafter a nearly constant urinary nitrogen excretion. Thus, adapting the animals a minimum of seven days to an isonitrogenous diet should allow the animals to come into nitrogen equilibrium and negate the influence of previous protein nutriture. As reported by Milner and Visek (1978), the protein content of the diet influences the excretion of orotate. This influence was very evident in this experiment. There was an extreme increase in orotic acid excretion in response to the -Arg diet during the first phase. However, there was little or no orotic aciduria in response to the second -Arg period. It was concluded that previous high protein intake (21%) during the adaptation resulted in the significant increase in orotic acid excretion during the first phase. During the second phase, the animals had essentially been adapted to a lower protein intake and this appeared to mute the orotic acid response to the second -Arg period. Due to the apparent impact of

nitrogen or protein metabolism on orotate excretion and thus urea cycle enzyme activity, it was also concluded that future TPN administered animals needed to have a recovery period after surgery to normalize the protein metabolism. This would allow the impact of arginine on urea cycle sufficiency to be studied without confounding the data due to animal variations in response to minor trauma. Popp et al. (1987) reported that a three-day post-surgery recovery period was sufficient to allow animals to return to baseline nitrogen turnover as well as a return of catecholamines to normal values.

The third experiment was designed as the intravenously-administered TPN counterpart of the previous experiment. The experiment was a crossover design in which the animals were divided into 5 groups. A control group received the unsupplemented lactalbumin diet, another group received first 0.78% arg and then 1.61% gln, a third group received first 1.61% gln and then 0.78% arg, the fourth group received first 3.23% arg and then 5.36% gln and the fifth group received first 5.36% gln and then 3.23% arg. Animals were adapted isonitrogenously to the unsupplemented lactalbumin diet and allowed a three day recovery period post-surgery during which time sterile saline was infused at approximately 15 mL/d. The animals were allowed to feed per os on the unsupplemented lactalbumin diet at the same rate as during the adaptation period. There was also a readaptation period between the first supplemented period

and the second -Arg period. A total of seven animals were assigned to the control group and a total of twenty animals were catheterized for TPN administration throughout the entire experiment. Only seven TPN animals survived to the end of the experiment. Throughout the experiment, the animals suffered mechanical complications of TPN administration. A total of nine (45%) TPN animals were euthanized due to severe diet infiltration and another four (20%) animals succumbed to anesthesia during the repair of either a catheter or assembly cup. A total of eleven (55%) animals had to have the assembly cup reattached to the back of their necks. At the termination of the experiment, catheter placement and tissue necrosis were determined. Catheter length from insertion site to tip was measured and there was a range from approximately 0.5 cm to 3.0 cm. A total of four TPN animals had dissolution of the vein in the vicinity of the catheter tip and diet leaking from the area. These animals also had slight to severe tissue necrosis around the dissolution site. A total of five animals had slight to severe lung emboli. The etiology of the emboli was not clear but it was associated with TPN feeding, as none of the control animals had lung emboli. One TPN administered animal had no tissue necrosis, leaking or any other visible sign of mechanical complication which would be associated with TPN administration. The catheter was inserted exactly 3.0 cm from insertion site and it was concluded that this distance would become the standard for

future TPN experiments. It was also concluded that the assembly cups were sutured too tightly to the neck, thus, a double-half hitch knot at each of the cup attachment sites would be used in future TPN experiments.

Organ weights at the end of the experiment are listed in Table 10. Urine volumes were elevated in the TPN administered animals (Figure 15). Urinary orotic acid excretion was extremely varied among animals on the same diet (Table 11), but the general trend of orotic acid excretion was an increase during -Arg administration and a decrease with +Arg administration. Glutamine supplementation did not appear to decrease orotic acid excretion to the same degree as arginine supplementation. However, due to the extremely large variation in animals within a group in response to the diets, statistical determination of dietary differences in orotic acid excretion are difficult to interpret. The one animal which did not have tissue abnormalities in the catheter insertion site area had an orotic acid excretion pattern which increased with -Arg administration and decreased with +Arg administration (Figure 16). Upon gln supplementation, the overall trend was an increase in orotic acid excretion. Due to the abnormal response in urinary orotic acid and severe loss of animals in the experimental groups which was

Table 10. Body and Organ Weights for Rats Administered TPN Diets Supplemented to Two Levels of Arginine and Two Levels of Glutamine and Control.

(number/group)	3.23% Arg (1)	5.38% Gln (2)	0.78% Arg (2) (means $\pm$ SD)	1.61% Gln (2)	Control (6)
Body Weight (g)					
	349.14	371.39 $\pm$ 22.05	332.64 $\pm$ 25.8	368.14 $\pm$ 28.5	361.32 $\pm$ 34.9
Organ Weight (g/100 g BW)					
Lung	0.67	0.76 $\pm$ 0.02	0.55 $\pm$ 0.01	0.70 $\pm$ 0.04	0.48 $\pm$ 0.04
Liver	3.34	3.80 $\pm$ 0.15	3.50 $\pm$ 0.16	3.35 $\pm$ 0.20	3.34 $\pm$ 0.20
Spleen	0.28	0.42 $\pm$ 0.06	0.32 $\pm$ 0.02	0.28 $\pm$ 0.01	0.22 $\pm$ 0.02
Kidney	0.76	0.74 $\pm$ 0.03	0.72 $\pm$ 0.04	0.69 $\pm$ 0.01	0.70 $\pm$ 0.02
Heart	0.41	0.39 $\pm$ 0.03	0.39 $\pm$ 0.05	0.34 $\pm$ 0.03	0.36 $\pm$ 0.02
GI*	3.50	3.44 $\pm$ 0.66	3.32 $\pm$ 0.61	3.26 $\pm$ 0.11	3.63 $\pm$ 0.44

* Small intestine from stomach to cecum with mesentery intact.

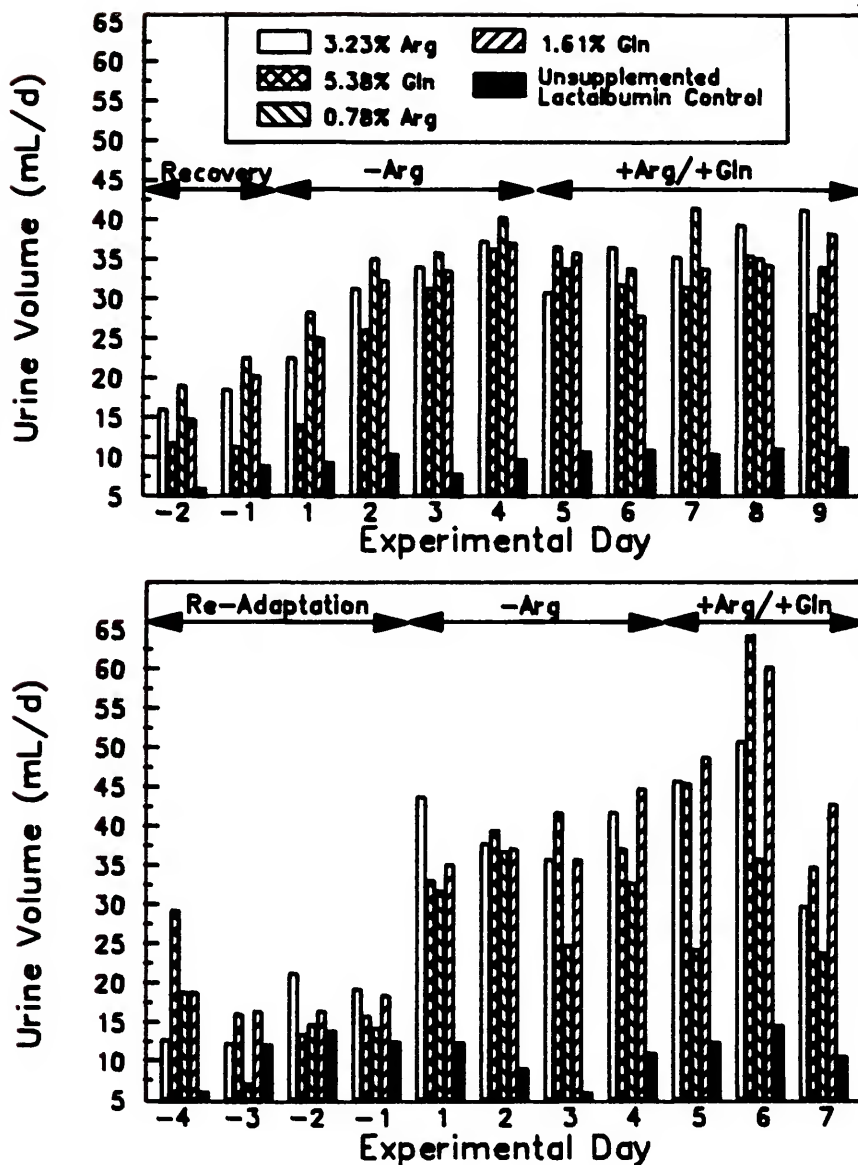


Figure 15. Average Daily Urine Volume of Rats Administered a TPN Formula Supplemented to One of Two Levels of Arginine, then, One of Two Levels of Glutamine and Control.

Table 11. Urinary Orotic Acid Excretion for Rats Administered TPN Diets Supplemented to Two Levels of Arginine and Two Levels of Glutamine and Control.

Day	3.23% Arg	5.38% Gln	0.78% Arg (means $\pm$ SD)	1.61% Gln	Control
-2	194.0 $\pm$ 13	586.6 $\pm$ 827	1182.8 $\pm$ 1733	838.9 $\pm$ 936	1580.3 $\pm$ 1278
-1	614.3 $\pm$ 513	253.1 $\pm$ 63	1570.3 $\pm$ 1591	1445.6 $\pm$ 1880	3057.4 $\pm$ 2688
1	342.2 $\pm$ 155	312.1 $\pm$ 27	1623.5 $\pm$ 1788	1988.2 $\pm$ 3000	1204.0 $\pm$ 1059
2	426.7 $\pm$ 495	232.0 $\pm$ 27	342.8 $\pm$ 184	621.9 $\pm$ 1042	1802.8 $\pm$ 1388
3	2822.2 $\pm$ 3491	1454.0 $\pm$ 1326	458.3 $\pm$ 288	1614.7 $\pm$ 2245	2208.2 $\pm$ 2307
4	1136.0 $\pm$ 1038	673.0 $\pm$ 413	1015.7 $\pm$ 1471	1356.3 $\pm$ 2424	1394.0 $\pm$ 1684
5	499.0 $\pm$ 331	1086.4 $\pm$ 1007	2868.2 $\pm$ 4539	1344.2 $\pm$ 2203	633.8 $\pm$ 849
6	1840.5 $\pm$ 1831	1671.4 $\pm$ 1363	870.8 $\pm$ 778	818.4 $\pm$ 1177	1188.0 $\pm$ 1301
7	722.3 $\pm$ 856	2209.6 $\pm$ 1712	1416.2 $\pm$ 1805	2119.0 $\pm$ 3552	611.5 $\pm$ 793
8	298.0 $\pm$ 41	883.3 $\pm$ 969	692.7 $\pm$ 669	346.0 $\pm$ 102	902.0 $\pm$ 1330
9	379.0 $\pm$ 140	838.7 $\pm$ 570	263.7 $\pm$ 20	891.5 $\pm$ 795.5	831.0 $\pm$ 796

Days -2 and -1 are the last two recovery days; Days 1 through 4 are -Arg and Days 5 through 9 are supplemented days at the level specified at the head of the column.

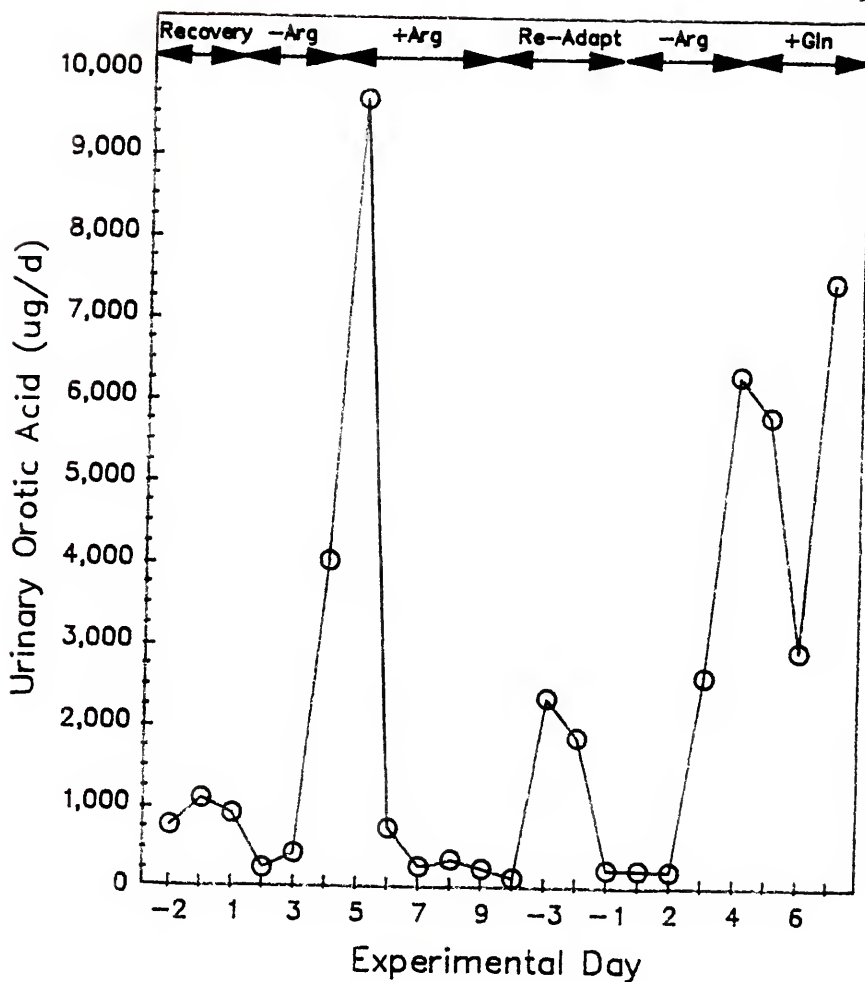


Figure 16. Daily Urinary Orotic Acid Excretion of an Animal to Which a TPN Formula Supplemented to 3.23% Arginine, then, 5.36% Glutamine was Successfully Administered Throughout the Twenty-Two Day Infusion.

apparently associated with catheter misplacement, no further urinary metabolites from this experiment were measured.

The overall conclusion of this experiment was that catheter position is critical for maintenance of animals; and this position was determined to be 3.0 ± 0.2 cm from the insertion site. Even though only one animal survived the entire 22-day experiment, it was concluded that the animal did respond in the predicted manner to -Arg and +Arg diets. The results from this animal provided further evidence that isonitrogenous replacement of arg by gln does not appear to provide sufficient arg precursors to be useful as a complete substitute for arg in TPN diets.

The next experiment was designed to test catheter placement and +Arg and +Gln supplementation at the higher levels in TPN administered rats. There were three groups, an unsupplemented lactalbumin control group, and two TPN groups; one received 3.27% arg and the other received 5.3% gln. Arginine or glutamine supplementation began after a four-day -Arg period. Sixteen rats were catheterized and divided evenly between the two TPN groups. A total of twelve TPN-administered rats, six per diet, completed the experiment. Six animals were assigned to the control group.

Final body weights and heart and kidney weights were not different among the three groups (Table 12). Lung weights were heavier in the TPN groups relative to the

Table 12. Final Body and Organ Weights for Rats Administered a Total Parenteral Nutrition Diet Containing Either 3.27% Arginine or 5.35% Glutamine.

	3.27% Arg	5.35% Gln (means $\pm$ SD)	Control
Final Body Weight (g)			
	268.53 $\pm$ 7.0 ^a	277.42 $\pm$ 14.1 ^a	284.57 $\pm$ 11.9 ^a
Organ Weights (g/100 g BW)			
Liver	3.88 $\pm$ 0.21 ^{a,b}	3.97 $\pm$ 0.21 ^a	3.64 $\pm$ 0.32 ^b
Kidney	0.68 $\pm$ 0.07 ^a	0.70 $\pm$ 0.05 ^a	0.75 $\pm$ 0.04 ^a
Spleen	0.37 $\pm$ 0.09 ^a	0.29 $\pm$ 0.03 ^b	0.25 $\pm$ 0.03 ^b
Lung	0.71 $\pm$ 0.08 ^a	0.77 $\pm$ 0.19 ^a	0.51 $\pm$ 0.04 ^b
Heart	0.41 $\pm$ 0.04 ^a	0.39 $\pm$ 0.03 ^a	0.38 $\pm$ 0.02 ^a
GI*	2.39 $\pm$ 0.18 ^b	2.66 $\pm$ 0.39 ^{a,b}	2.91 $\pm$ 0.28 ^b

* Small intestine from stomach to cecum with mesentery intact.

Values without a common superscript within a row differ by $p < 0.05$.

control group ($p \leq 0.05$). Spleen weight was heavier in the +Arg group relative to the two other groups ($p \leq 0.05$). This concurs with data presented by Popp et al. (1982, 1984). It helps substantiate the theory that immune stimulation due to arginine supplementation in TPN is responsible for heavier splenic weights, rather than splenic congestion. There was an overlapping in differences between dietary groups for GI and liver, thus no conclusion could be made regarding the effect of diet on these organs.

Urine volume was not different between the three groups for the first two days of surgery recovery (experimental days -3 and -2). However, as the infusion rate increased in the TPN groups, the urine volume of these two groups was statistically higher than controls but was not different between the two TPN groups ($p \leq 0.05$) (Figure 17).

The TPN rats did not exhibit an increase in orotic acid excretion during the -Arg period relative to control rats or within the respective dietary group during the recovery period (Figure 18). Upon supplementation of 3.27% arg, the rats exhibited a two-fold drop in orotic acid excretion. After three days of +Arg, the rats in this dietary group were excreting less orotic acid than during the recovery period or -Arg period ($p \leq 0.05$). After four days of +Arg, this group was excreting slightly less than 200 $\mu\text{g/d}$ orotic acid. This value was double that which had been reported by

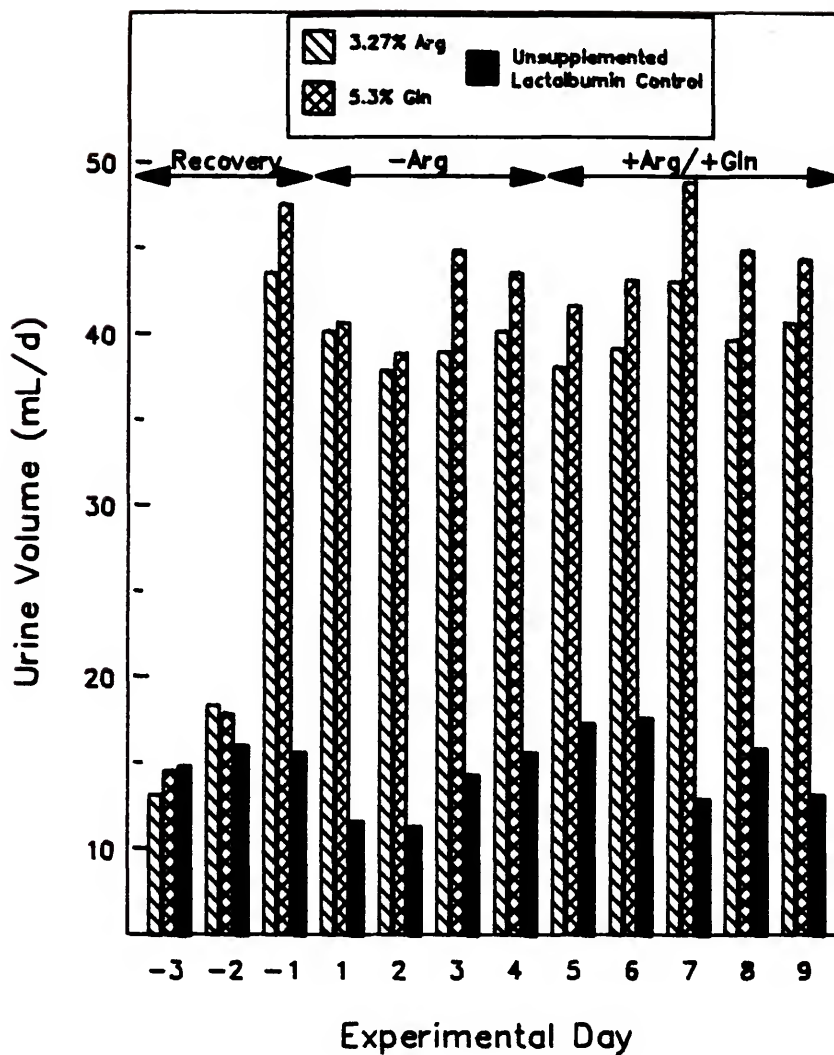


Figure 17. Average Daily Urine Volumes of Rats Administered a TPN Formula Supplemented to Either 3.27% Arginine or 5.3% Glutamine and Controls.

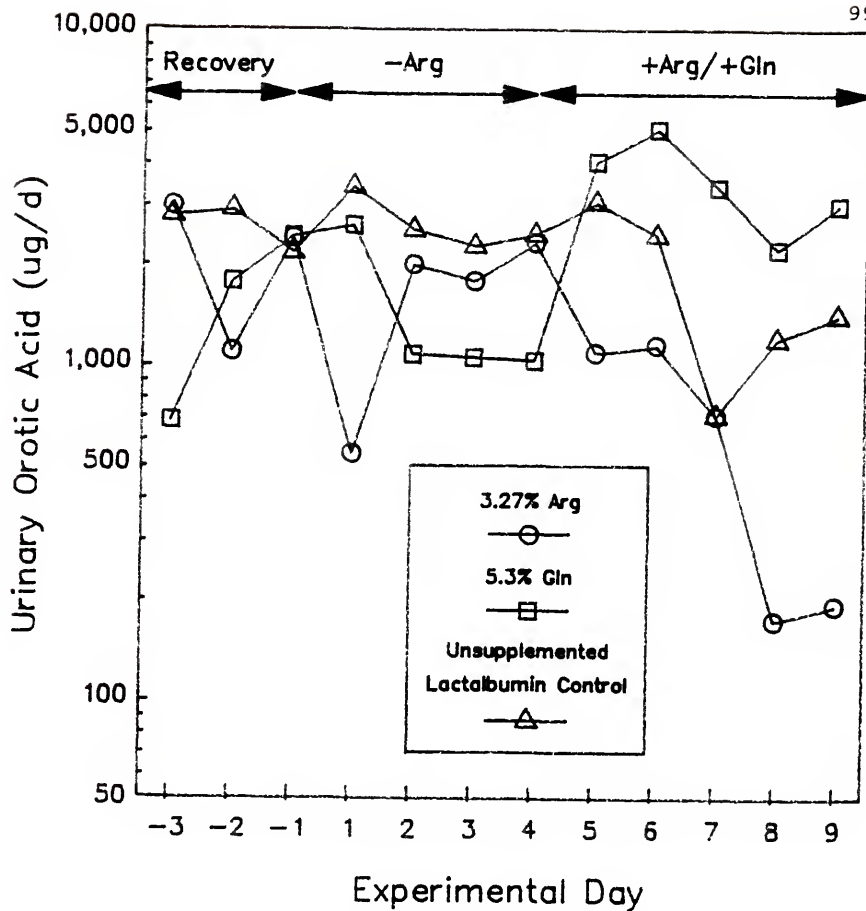


Figure 18. Average Daily Urinary Orotic Acid Excretion of Rats Administered a TPN Formula Supplemented to Either 3.27% Arginine or 5.3% Glutamine and Controls.

Hassan and Milner (1979) as control values for 180-200 g rats on a 14% L-amino acid diet. The +Gln group did not exhibit a decrease in orotic acid excretion upon supplementation. This gave further evidence that gln cannot provide isonitrogenous replacement for arginine in a TPN formulation. Control animals excreted between 1000 and 3000 μg orotic acid/day throughout the entire experiment. It was concluded that the protein source, lactalbumin, did not contain sufficient arginine to permit orotic acid excretion levels within the normal range of 100 $\mu\text{g}/\text{d}$. Amino acid analysis of the lactalbumin hydrolysate revealed that lactalbumin contained 2.8% arginine.

There was no statistical difference in citrate excretion between the three groups on the second or third day of surgery recovery (Figure 19). Upon -Arg administration, the TPN groups excreted statistically less citrate than controls. Upon arg or gln supplementation, citrate excretion rose the first two days. Except for the first and fourth day of supplementation, there was no difference in citrate excretion between the three groups ($p \leq 0.05$). On Day 5 (first day of supplementation), there was no difference in citrate excretion between the 3.27% arg group and the control. However, on the fourth day of supplementation (Day 8), the 3.27% arg group excreted the most citric acid ($p \leq 0.05$).

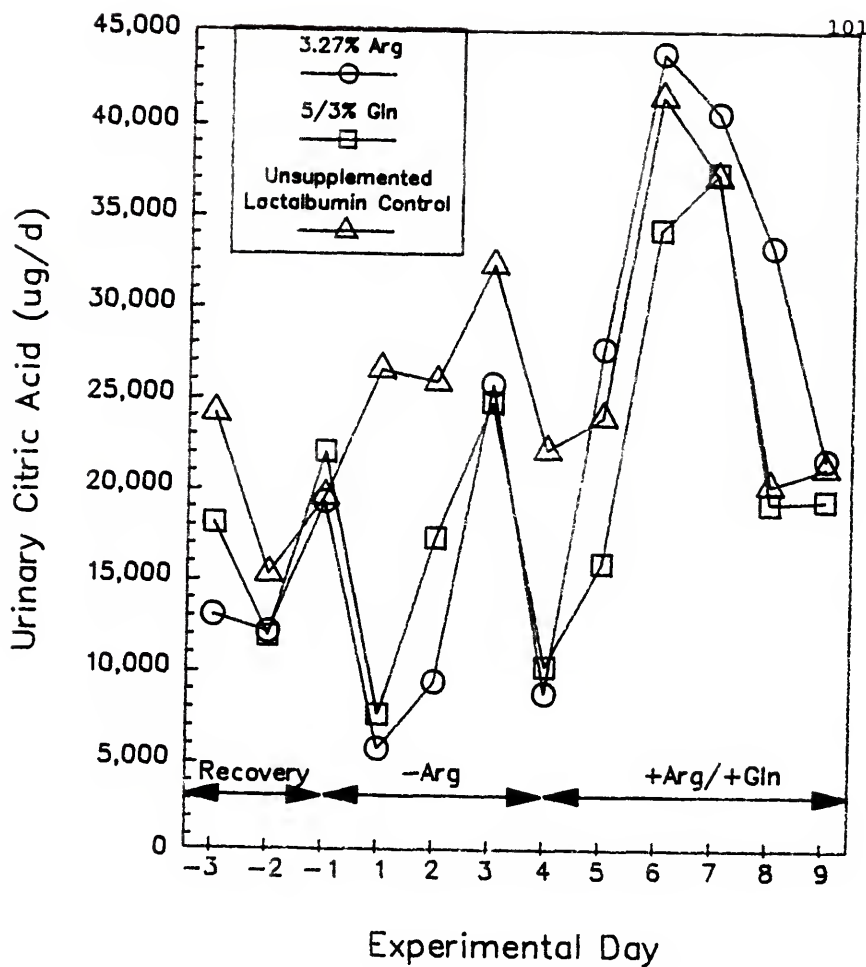


Figure 19. Average Daily Urinary Citric Acid Excretion of Rats Administered a TPN Formula Supplemented to Either 3.27% Arginine or 5.3% Glutamine and Controls.

The two TPN groups had statistically higher UUN excretion during the first two days of surgical recovery, relative to the control rats (Figure 20). Upon -Arg administration, the two TPN groups began excreting less UUN than controls; this continued throughout the remainder of the experiment. There was no difference in UUN excretion between the two TPN groups throughout the entire experiment. The decreased UUN excretion in the TPN administered animals is indicative of an increased nitrogen retention by the animals.

Only on Days -1 and 7 was there a statistical difference in creatinine excretion between the three groups (Figure 21). Individual animal CV for creatinine excretion varied between 9.5 and 18.5% which is within reported normal daily variation in creatinine excretion. Thus it is assumed that complete urine collections were made.

The conclusions from this experiment were that a supplementation level of 3.25% arg is near the range of adequacy, lactalbumin must be supplemented with arginine to provide for sufficient urea cycle operation and that glutamine cannot provide a complete replacement for arginine in a TPN formula.

To determine the level of arginine supplementation needed in the lactalbumin diet, 2 rats each were fed a lactalbumin diet containing 5.0, 6.5 or 8.0% arginine as a percent of total protein for a period of four days. On day

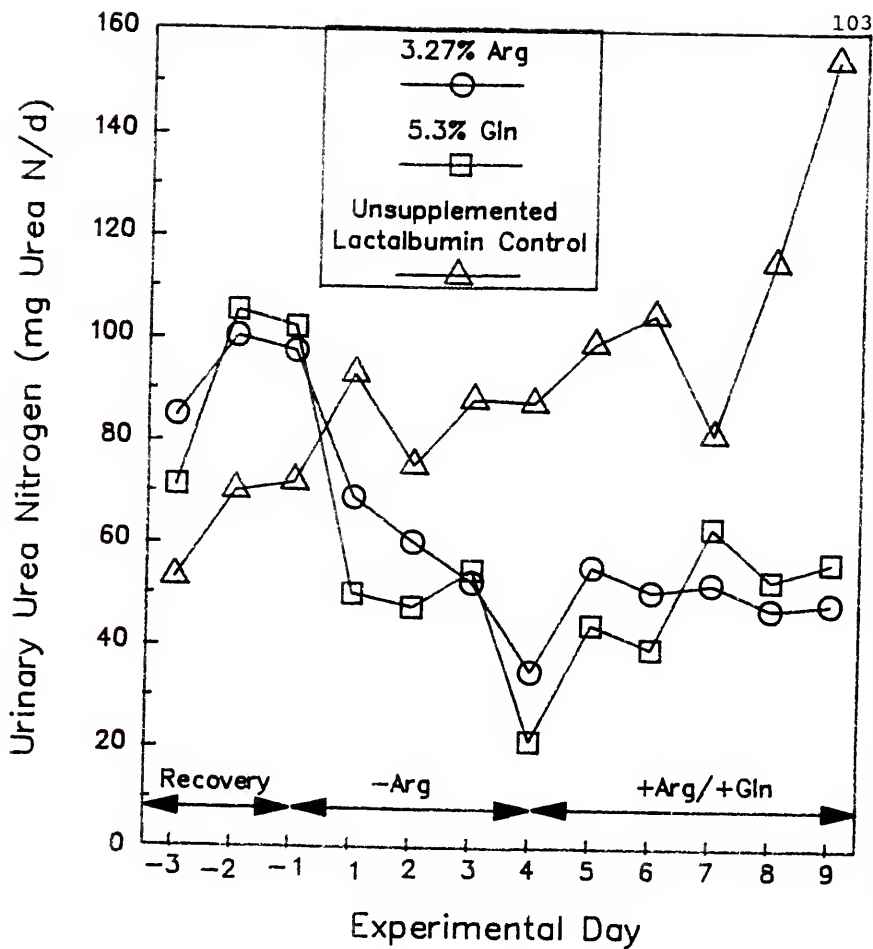


Figure 20. Average Daily Urinary Urea Nitrogen Excretion of Rats Administered a TPN Formula Supplemented to Either 3.27% Arginine or 5.3% Glutamine and Controls.

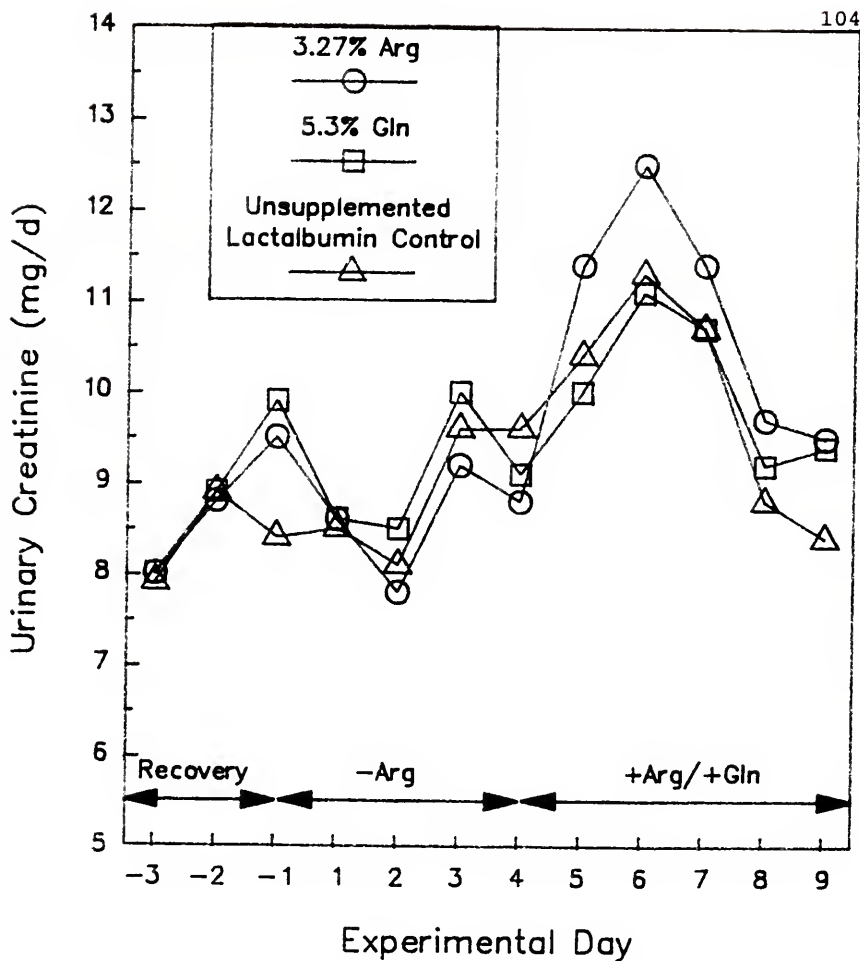


Figure 21. Average Daily Urinary Creatinine Excretion of Rats Administered a TPN Formula Supplemented to Either 3.27% Arginine or 5.3% Glutamine and Controls.

four, twenty-four-hour urine collections were made and urinary orotate measured. The rats consuming the diet supplemented to 6.5% arginine of total protein excreted 98 and 105 g orotate. It was concluded that the rats would be fed a 6.5% arg supplemented lactalbumin diet in future experiments.

The conclusions relative to this objective were as follows:

1. Catheters must be inserted to 3.0 ± 0.2 cm in animals weighing 275 ± 25 g.
2. Lactalbumin adaptation and control diet must be supplemented to 6.5% arg to total protein.
3. An arginine supplementation level around 3.25% is near adequacy for TPN administered 275-300 g rats given 290 mg N per day and 60 non-N Kcal per day.

Determination of Arginine Adequacy in TPN

The response of adult rats (250 g) maintained on TPN containing approximately 1.6, 2.5, 3.2 and 4.8% arg after a 4-day -Arg infusion was determined. The actual percent arg in the diets was determined by automated amino acid analysis and the amounts were 1.76, 2.64, 3.42 and 5.14%. The experimental design consisted of the 1.76 and 3.42% TPN groups and a supplemented lactalbumin control group designated as Control I in the first time period, and the

2.64 and 5.14% TPN groups and a supplemented lactalbumin control group designated as Control II in the second time period.

Table 13 lists body weight, weight gain per day and excised organs. Final body weights (with initial BW as a covariate) did not show statistical differences between groups except the Control I, which was different from the TPN groups. Weight gain/day was not statistically different among any of the groups ($p \leq 0.05$). Popp et al. (1982, 1984) have shown that TPN-fed rats infused with an adequate diet increase weight at the same rate as orally fed rats. Thus, it can be assumed that the TPN-fed animals of these experiments and the lactalbumin-fed animals had equivalent nutrition.

The kidney was the only organ which did not show a significant difference in weight between all groups. Lung, liver and spleen weights were significantly heavier in the TPN groups relative to control animals. Analysis of variance analysis of heart weight showed a moderate significant difference between dietary groups ($p \leq 0.020$). Duncan's analysis of the mean heart weights for the dietary groups yielded an overlapping of nonsignificance between all diets, thus, physiologically there may not be a difference in heart weight due to diet. GI weight in TPN administered animals was significantly lower relative to control animals. This is a well documented characteristic of TPN feeding

Table 13. Body Weights and Organ Weights for Rats Administered Graded Levels of Arginine by TPN.

Group (n)	BW ¹ (g)	Wt gain ² (g/day)	Lung	Liver	Spleen (g/100g BW)	Kidney	Heart	GI
1.76% (5)	311.88 ^b ±15.1	3.07 ^a ±0.81	0.60 ^b ±0.1	3.17 ^{bc} ±0.23	0.32 ^{cd} ±0.05	0.64 ^a ±0.05	0.36 ^{abc} ±0.05	1.44 ^c ±0.09
2.64% (6)	306.41 ^b ±14.0	2.81 ^a ±1.05	0.92 ^a ±0.27	3.89 ^a ±0.43	0.56 ^a ±0.1	0.67 ^a ±0.05	0.4 ^a ±0.05	1.68 ^{ab} ±0.17
3.42% (6)	308.98 ^b ±14.1	3.13 ^a ±1.88	0.59 ^{bc} ±0.04	3.52 ^{ab} ±0.44	0.38 ^{bc} ±0.03	0.66 ^a ±0.05	0.34 ^{bc} ±0.02	1.42 ^c ±0.24
5.14% (6)	313.30 ^b ±13.3	3.31 ^a ±0.43	0.78 ^a ±0.16	3.41 ^b ±0.25	0.44 ^b ±0.16	0.62 ^a ±0.03	0.38 ^{ab} ±0.02	1.62 ^{bc} ±0.22
Control I (6)	342.91 ^a ±13.1	3.45 ^a ±0.33	0.41 ^c ±0.03	2.93 ^c ±0.13	0.22 ^d ±0.01	0.62 ^a ±0.03	0.33 ^c ±0.02	1.87 ^a ±0.18
Control II (6)	327.99 ^{ab} ±13.3	3.85 ^a ±0.60	0.41 ^c ±0.01	3.28 ^{bc} ±0.35	0.21 ^d ±0.03	0.62 ^a ±0.05	0.38 ^{ab} ±0.03	1.91 ^a ±0.09

means ± SD without common superscript within a column differ at $p \leq 0.05$

¹ LSmean with initial weight as a covariate.

² determined from fasted body weight for TPN animals and non-fasted weight for Lactalbumin control animals

because of disuse atrophy of the GI tract (Hughes & Dowling, 1980).

Significant organ weight differences among the TPN administered animals were not entirely comparable to data obtained by Popp et al. (1982, 1984). Popp et al. reported significantly heavier spleen and kidney weights in TPN-fed animals relative to semipurified diet-fed controls. Although their data for lung and liver did not show statistically significant differences, the trend was toward heavier weights in the TPN animals. Increased spleen weight is a common observation in TPN-fed animals. Popp et al. attributed this to either immune stimulation or splenic congestion, the mechanism of which was unclear.

Moisture and total nitrogen was determined on the organs shown in Table 14 from the second time period experimental groups. There was no difference in lung and liver moisture content between the 2.64% and Control II groups, but both groups were higher ($p \leq 0.05$) in moisture than the 5.14% group. Spleen moisture content was not different between the two TPN groups and both were higher in moisture than Control II. The spleen moisture of the 5.14% group was also not different from that of the Control II group. Thus, excess water retention does not appear to be a contributing factor in the increased weight for lung or liver. However, excess water/fluid retention in the spleen may be a contributing factor for the increased spleen weight

Table 14. Moisture and Protein Content of Organs from Rats Fed Either 2.64% Arg or 5.14% Arg Supplemented TPN and Control.

Group	Lung	Liver	Spleen
Moisture Content (%)			
2.64%	78.33 $\pm$ 0.4 ^a	72.48 $\pm$ 0.6 ^a	78.93 $\pm$ 0.6 ^a
5.14%	76.67 $\pm$ 1.3 ^b	71.08 $\pm$ 1.0 ^b	78.28 $\pm$ 0.7 ^{a,b}
Control II	79.33 $\pm$ 0.6 ^a	72.73 $\pm$ 0.9 ^a	77.59 $\pm$ 0.8 ^b
Total Nitrogen (mg N/whole tissue)			
2.64%	51.78 $\pm$ 13.2 ^a	284.74 $\pm$ 26.0 ^a	40.59 $\pm$ 10.7 ^a
5.14%	47.85 $\pm$ 8.2 ^a	249.29 $\pm$ 29.8 ^b	33.26 $\pm$ 11.4 ^a
Control II	25.57 $\pm$ 1.9 ^b	286.48 $\pm$ 19.2 ^a	17.96 $\pm$ 2.3 ^b

Values are mean $\pm$ SD

Those without a common superscript within a column differ at $p \leq 0.05$.

in TPN-fed animals. Total nitrogen in lung and spleen were statistically different in the TPN-fed animals relative to the lactalbumin-fed animals. When data are expressed as mg N/g dry tissue however, there is no statistical difference in lung total nitrogen among the three groups. Liver total N was lowest in the 5.14% group and was different ($p \leq 0.05$) from the 2.64% TPN group and the Control II group. The data indicate that the heavier lung and liver weights in TPN-fed animals were due to organ hypertrophy rather than fluid retention. Increased spleen weight may be a combination of both increased hypertrophy and increased fluid retention.

Figure 22 shows orotic acid excretion for all diets over the course of the experimental period. All TPN groups excreted an average of 160.11 μg orotate/day during the recovery period (Days -3 to -1). All TPN groups exhibited orotic aciduria with the initiation of the -Arg TPN formula (Day 1). Day 1 of -Arg administration produced a range of 5- to 40-fold increase in orotic acid excretion. All TPN groups had at least a 30-fold increase in orotic acid excretion at some time during the -Arg period. Within 48 hours of arginine supplementation, all TPN groups except 1.76% excreted less than 200 μg orotate/day. Only the 2.64% arg group excreted orotic acid to levels which fluctuated above 200 μg /day after 48 hours of +Arg infusion. In both control groups, orotic acid values fluctuated between 100 and 200 μg orotate/day throughout the entire experimental

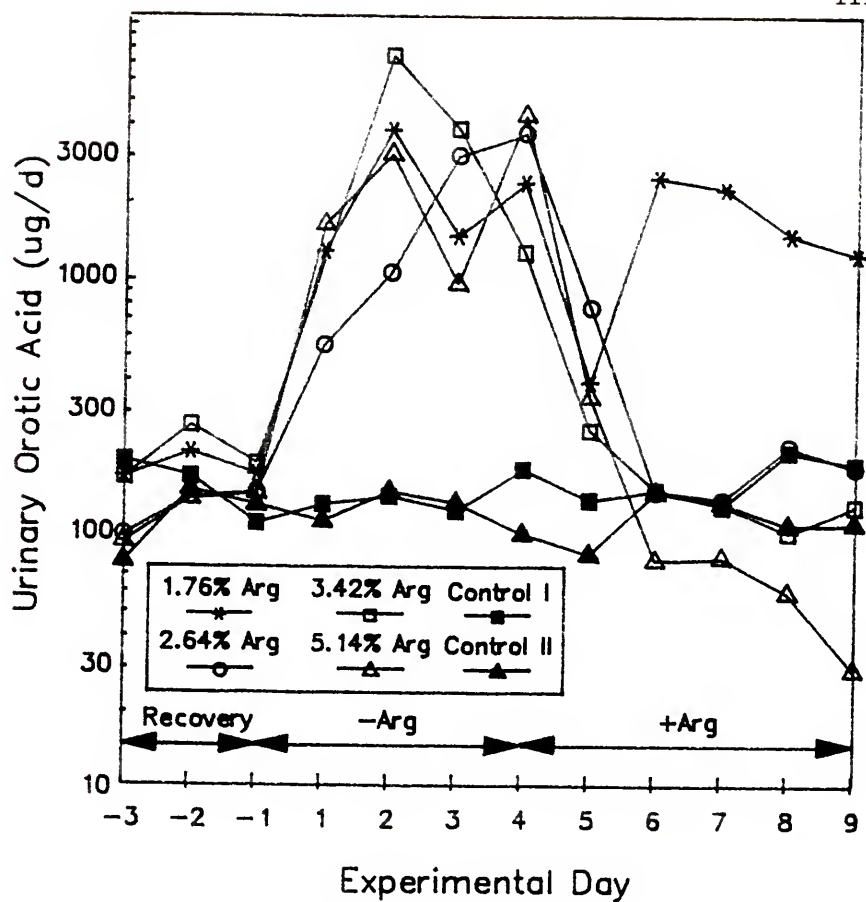


Figure 22. Average Daily Urinary Orotic Acid Excretion of Rats Administered a TPN Formula Supplemented With Graded Levels of Arginine and Controls.

period with an overall average of $120.87 \pm 21.2 \mu\text{g/d}$. After 48 hours of infusion, there was little or no difference in the daily orotate excretion levels for the 2.64, 3.42 and 5.14% arg diets, (Figure 23). Figure 24 shows the regression line for this time period. The equation is $[\text{orotate}] = -38.575x + 259.49$ with an $R^2 = 0.8250$, $p = 0.0001$. Assuming that an excretion of $100 \mu\text{g}$ orotate/day indicates arginine adequacy, then the calculated concentration of arginine to provide this level is $4.13\% \pm 0.16$.

Figure 25 depicts urinary urea nitrogen/100 g BW and Figure 26 depicts urinary total nitrogen/100 g BW over the entire experimental period. At the onset of the recovery period, there was a 2-fold increase in urinary urea nitrogen in all TPN groups relative to the controls. This is indicative of the metabolic response to the surgery of the previous day. Groups 2.64% and 5.14% appear to have become metabolically stable by Day -1 or the day prior to -Arg TPN administration as evidenced by urinary urea and total nitrogen excretion levels near those of controls. Groups 1.76% and 3.42% did not show a decrease in nitrogen during the 3-day recovery period. This may have been due to mechanical problems encountered at the beginning of this experiment with separation at the catheter-transmission connection. Administration of the -Arg formula resulted in an increase in both forms of nitrogen excretion for groups

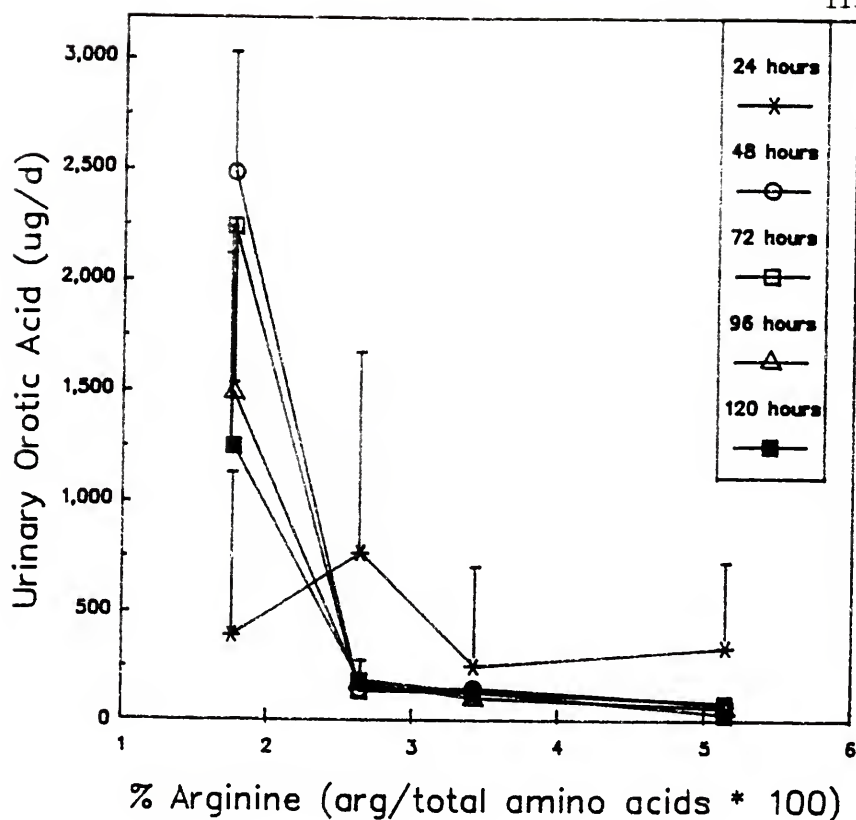


Figure 23. Dose Response Curve of Urinary Orotic Acid Excretion to Percent Arginine Supplementation for Rats Administered a TPN Formula Supplemented with Graded Levels of Arginine Between Twenty-Four and One Hundred and Twenty Hours of Supplemented Infusion.

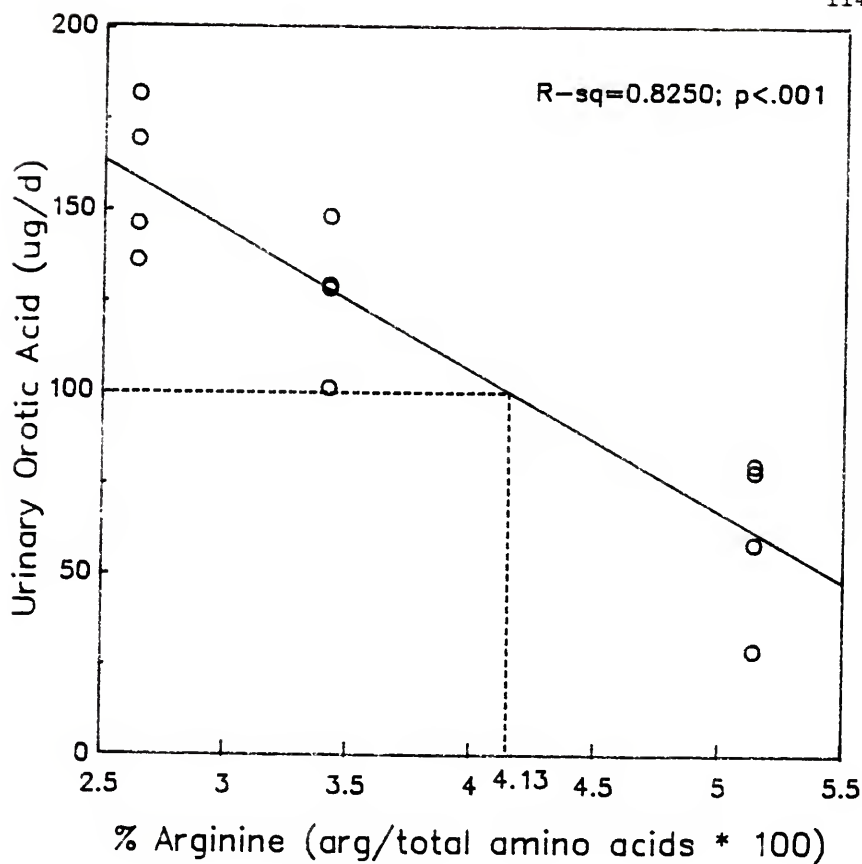


Figure 24. Regression Line of Urinary Orotic Acid Excretion versus Percent Arginine Supplementation for Rats Administered a TPN Formula Supplemented to Either 2.64%, 3.42% or 5.14% Arginine per Total Amino Acids in the TPN Formula.

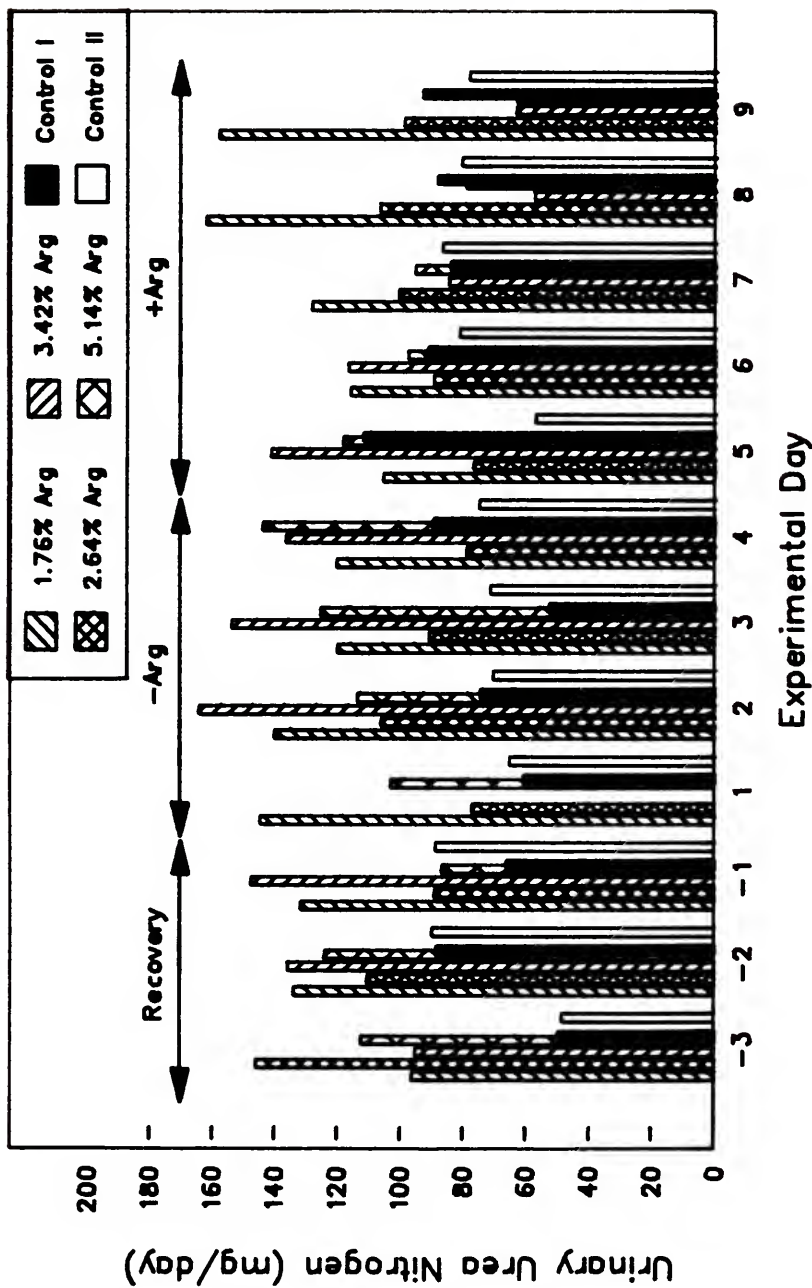


Figure 25. Average Daily Urinary Urea Nitrogen Excretion of Rats Administered a TPN Formula Supplemented with Graded Levels of Arginine and Their Respective Controls.

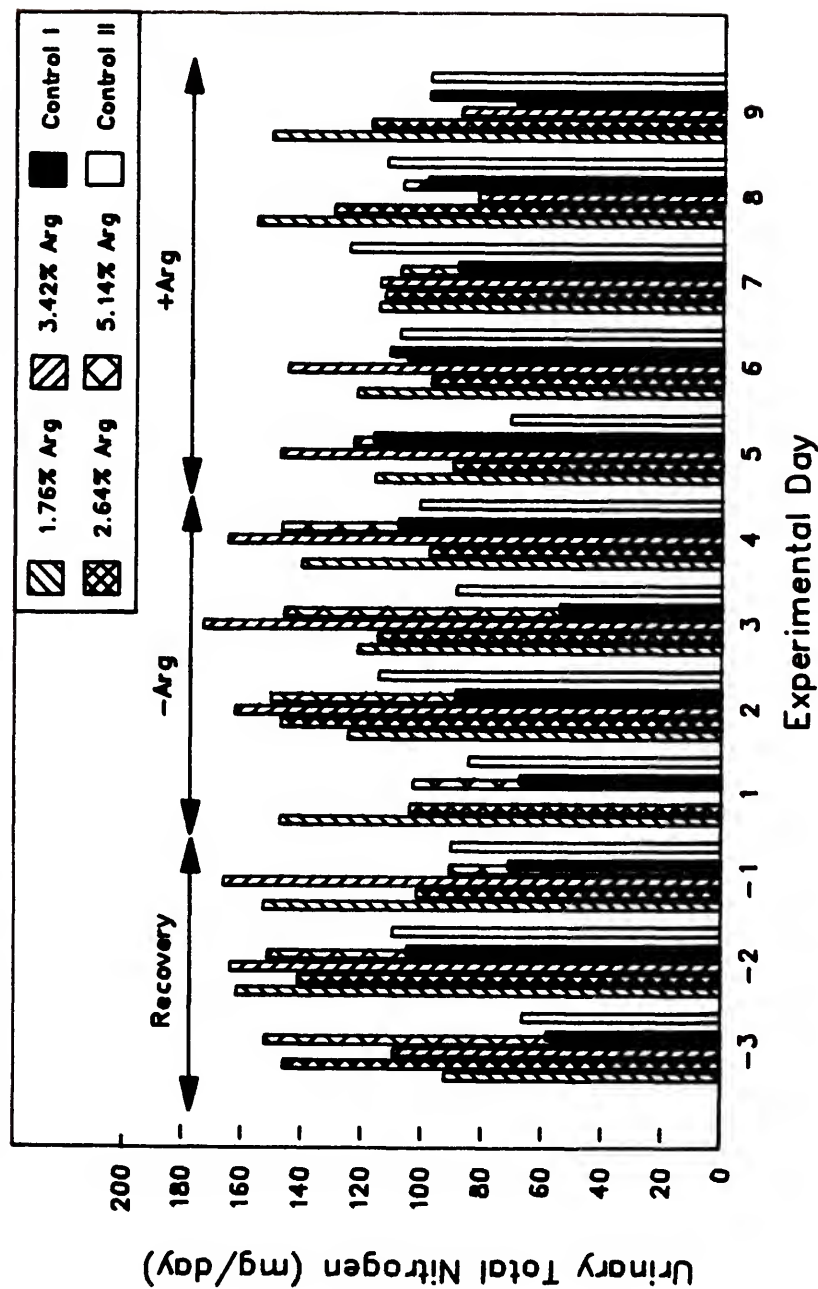


Figure 26. Average Daily Urinary Total Nitrogen Excretion of Rats Administered a TPN Formula Supplemented with Graded Levels of Arginine and Their Respective Controls.

2.64% and 5.14% and a sustained increased excretion for groups 1.76% and 3.42%. Upon arg supplementation, both 1.76% and 2.64% TPN groups showed increased levels of urinary urea and total nitrogen excretion. The 3.42% and 5.14% groups exhibited a decrease in both types of nitrogen excretion. According to Milner and Visek, adequate dietary arg supplementation demonstrates decreased levels of urinary urea nitrogen excretion. Thus, only the higher two levels of supplementation can be considered to be within the adequate range. It should also be noted that both the 3.42% and 5.14% arg levels showed lower urinary urea and urinary nitrogen excretion by the last day of arg supplementation relative to the lactalbumin controls. Milner and Visek (1973, 1978) demonstrated 5- to 10-fold increases in urinary urea nitrogen in immature rats upon administration of an arg devoid diet. The data from this experiment do not demonstrate this dramatic increase in urinary urea during the arg devoid period; this may be due to the maturity of the animals as well as the pretreatment adaptation to a restricted nitrogen intake.

Orotate and nitrogen excretion are interrelated by way of the urea cycle. As excess nitrogen enters the liver (predominately as gln or NH_3), the urea cycle enzymes convert this NH_3 to urea. In a situation in which arginine is inadequate, carbamoyl phosphate (CP) is synthesized in greater quantities than ornithine availability within the

mitochondria. The excess CP is 'transported' into the cytosol and is incorporated into pyrimidine biosynthesis. As a result, more orotic acid is formed than can be utilized and the excess is excreted in the urine. Under adequate dietary conditions, the orotic acid/nitrogen ratio in the urine should be low. Elevated ratios indicate inadequate nitrogen disposal capabilities. Figures 27 and 28 show 40 to 50-fold increases in orotic acid/urinary urea nitrogen and 30 to 45-fold increases in orotic acid/total urinary nitrogen during the arg devoid period, respectively, thus indicating impaired nitrogen disposal capacity. Arginine supplementation decreased these ratios to control levels within 48 hours of supplementation in all TPN groups except 1.76%. These graphs clearly indicate the inadequacy of 1.76% diet. In both graphs, 2.64% and 3.42% diets had slightly higher orotate/nitrogen ratios than the lactalbumin control. The 5.14% group on the other hand, had the lowest ratio of all groups. These data give further evidence that arginine adequacy in TPN for the specified conditions lies within 2.64% and 5.14% arg.

Creatinine excretion was used as a measure of completeness of urine collection within a 24-hr time period since creatinine excretion is thought to be relatively constant and not subject to variation due to dietary protein manipulation (Folin, 1905). Fisher (1965), however, demonstrated significant variations in creatinine excretion

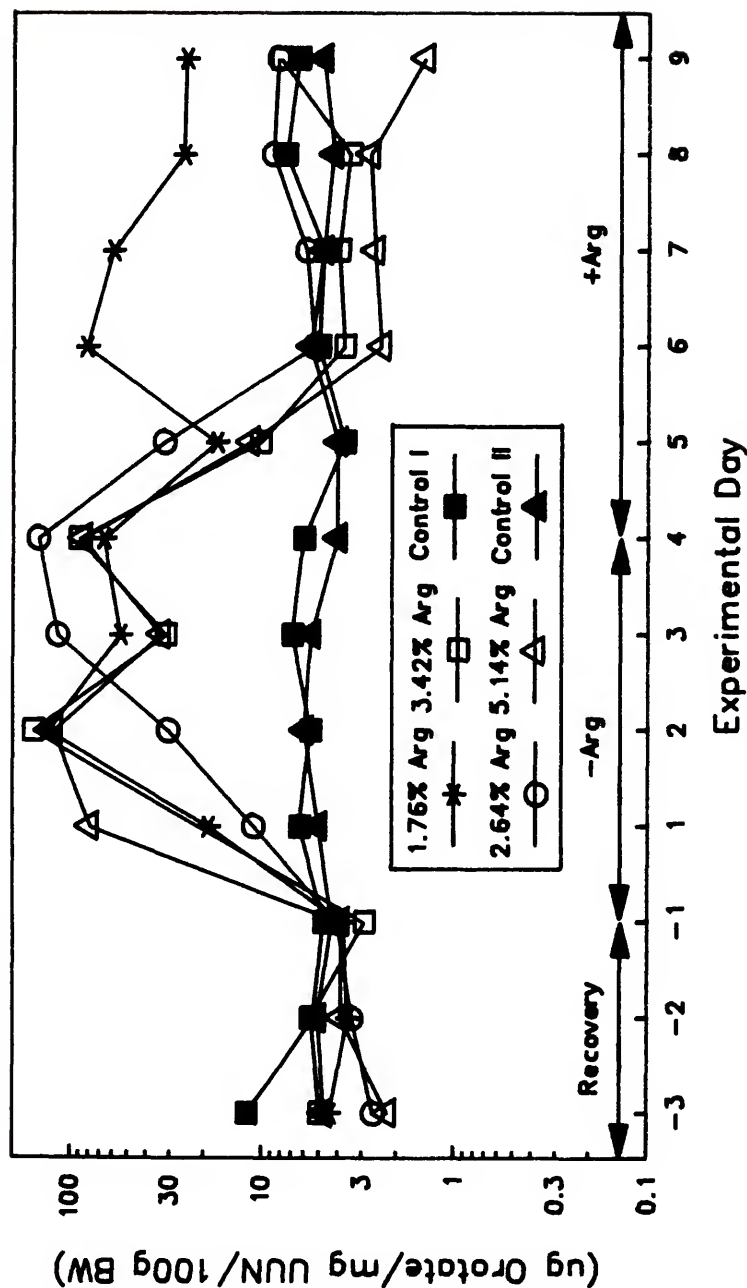


Figure 27. Ratio of Average Daily Urinary Excretion of Orotic Acid to Urea Nitrogen of Rats Administered a TPN Formula Supplemented with Graded Levels of Arginine and Their Respective Controls.

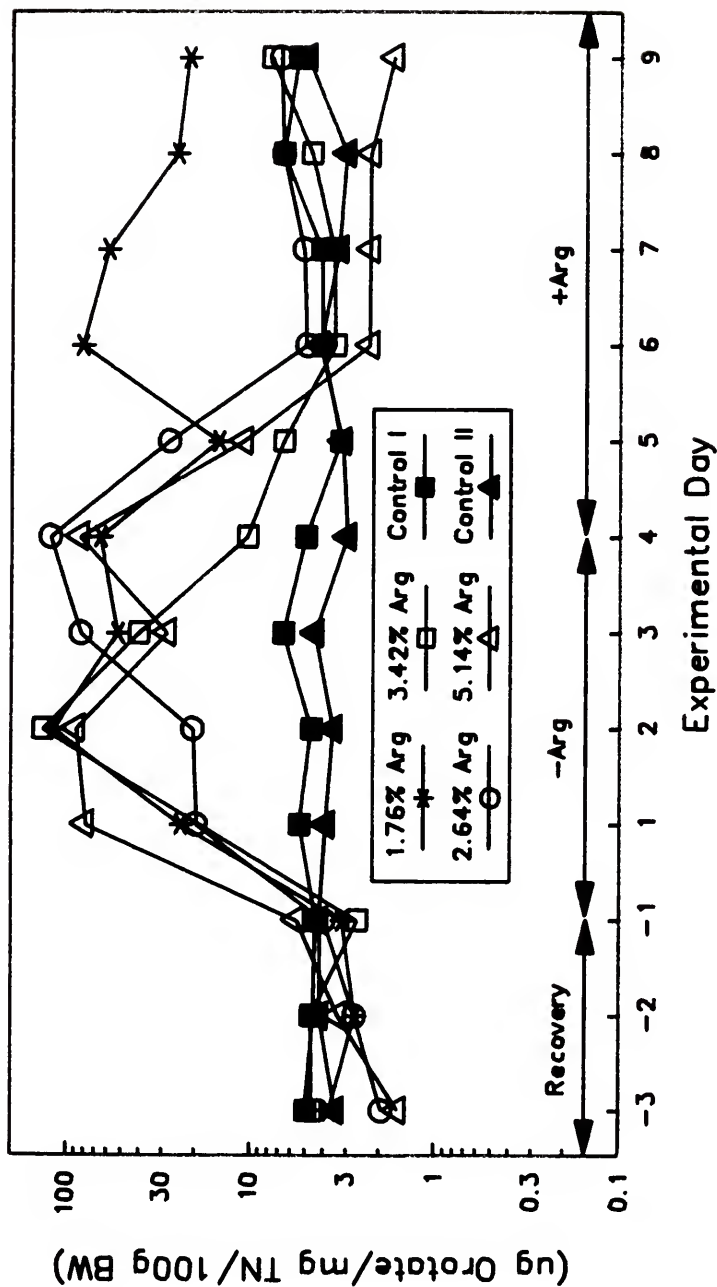


Figure 28. Ratio of Average Daily Urinary Excretion of Orotic Acid to Urinary Total Nitrogen of Rats Administered a TPN Formula Supplemented with Graded Levels of Arginine and Their Respective Controls.

with dietary protein manipulation. Fisher reported a coefficient of variation (CV) of daily urinary creatinine excretion between 10 and 20% for animals maintained on a 15% protein diet. This CV was increased to as much as 73% following a major change in dietary protein. Urinary creatinine excretion is depicted in Figure 29. Individual animal daily CV in this study showed an 8% CV for animals in the animals in the groups of the first time period and a 21% CV for animals in the second time period. The analysis of variance of daily creatinine variation showed highly significant differences among the diets with p values less than 0.001. However, Duncan's analysis of the TPN dietary group means showed differences ($p \leq 0.05$) between the recovery period (Days -3 to -1) and the first day of -Arg TPN administration (Day 1), but no difference in creatinine excretion after Day 1. Thus, it can be assumed that urine collections were complete. The lower urinary creatinine excretion on observed on Day -3 in the control groups was probably due to the overnight fast and transfer into metabolism cages. Within respective experimental time periods, there was no difference in creatinine excretion due to diet after the first day of -Arg administration.

All TPN groups, except the 1.76% group, excreted low levels of citrate on the day prior to TPN administration (Day -1). On the last day of -Arg administration (Day 4), all TPN groups excreted increased levels of citrate relative

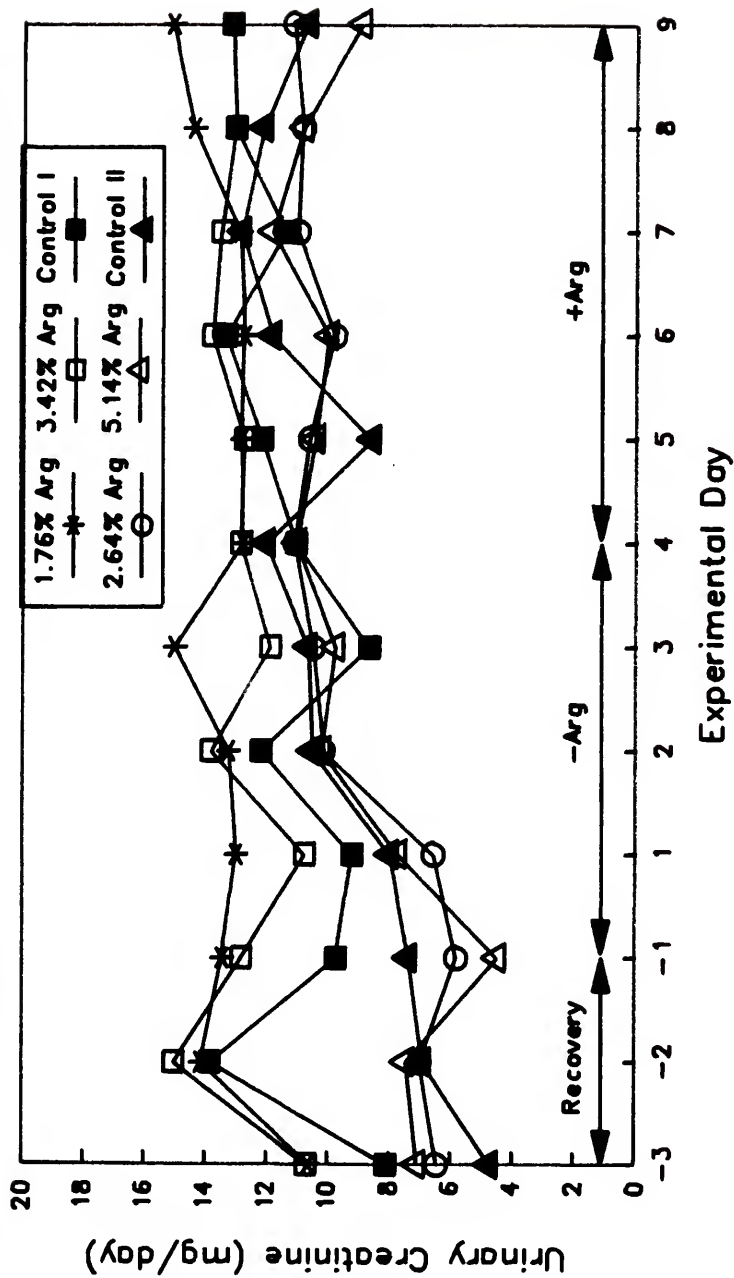


Figure 29. Average Daily Urinary Creatinine Excretion of Rats Administered a TPN Formula Supplemented with Graded Levels of Arginine and Their Respective Controls.

to controls and the previous analysis date (Figure 30). At the end of arg supplementation, only the 5.14% TPN group had decreased levels of citrate excretion. These data are in agreement with the findings of other investigators; urinary citrate was the only organic acid shown to increase due to a lack of arginine (Milner et al., 1975). Citrate excretion has been shown to increase with a systemic alkalosis and without a required concomitant increase in urinary pH (Simpson, 1983). Simpson concluded that the increase in citrate excretion resulted from a diminished citric acid cycle capacity and a decreased ability of the mitochondria to maintain a citrate gradient due to increased cytosolic concentrations.

Plasma amino acid concentrations are listed in Table 15. There was no statistical difference in plasma amino acid concentrations among the groups for pro, phe, val and his. Concentrations of cys and orn were different for only one group. The 3.42% arg group had a lower cys concentration ($p \leq 0.05$) and Control II had an elevated concentration of orn relative to all other groups. Duncan's analysis of dietary group means for glu, gln, ala, met and ile resulted in much overlapping of nonsignificance. Differences of asp and trp plasma concentrations among the dietary groups appeared to be due to the experimental period rather than a dietary effect. Aspartate and thr concentrations were not different between the dietary groups

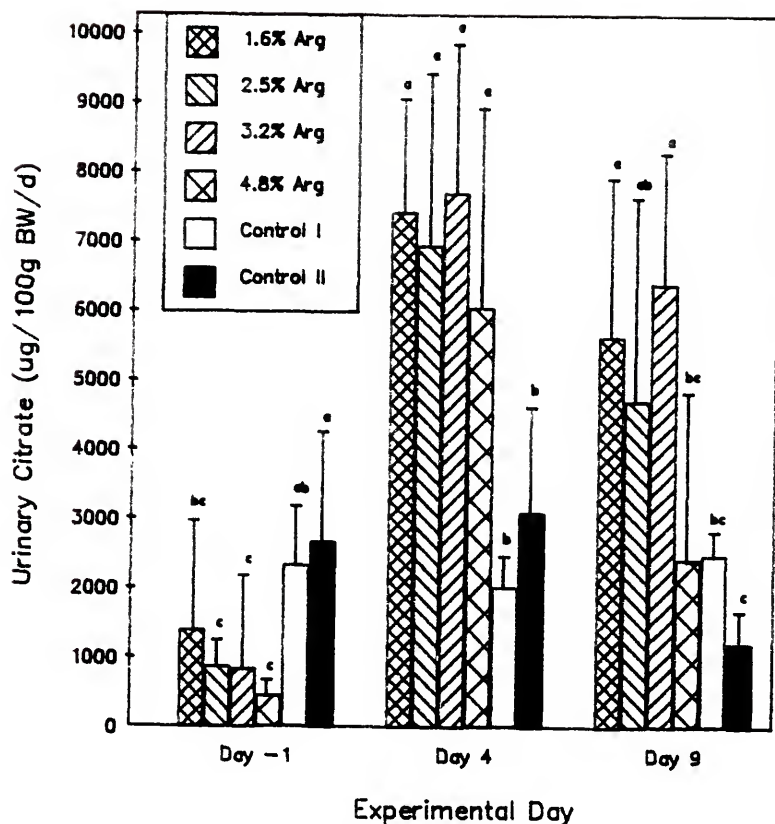


Figure 30. Average Urinary Citric Acid Excretion of Rats After a Three-Day Surgical Recovery Period, a Four-Day Arginine-Devoid TPN Administration and a Five-Day Administration of a TPN Formula Supplemented with Graded Levels of Arginine and Controls.

Table 15. Summary of Plasma Amino Acid Concentrations for Rats Fed Graded Levels of Arginine by TPN and Controls.

A.A.	1.76%	2.64%	3.42%	5.14%	Control I	Control II
Asp	17.8 $\pm$ 5 ^b	28.5 $\pm$ 8 ^a	20.1 $\pm$ 5 ^b	30.5 $\pm$ 5 ^a	16.4 $\pm$ 2 ^b	36.1 $\pm$ 10 ^a
Thr	369.7 $\pm$ 56 ^{ab}	307.5 $\pm$ 79 ^b	214.4 $\pm$ 57 ^c	285.3 $\pm$ 73 ^{bc}	412.0 $\pm$ 38 ^a	456.5 $\pm$ 86 ^a
Ser	763.84 $\pm$ 153 ^a	670.0 $\pm$ 181 ^{ab}	821.2 $\pm$ 188 ^a	839.2 $\pm$ 136 ^a	506.2 $\pm$ 55 ^b	552.1 $\pm$ 79 ^b
Asn	83.3 ^b	102.1 $\pm$ 25 ^b	119.4 $\pm$ 34 ^b	95.5 $\pm$ 18 ^b	171.5 $\pm$ 17 ^a	212.1 $\pm$ 38 ^a
Glu	202.6 ^b	261.0 $\pm$ 56 ^{ab}	281.6 $\pm$ 34 ^a	271.3 $\pm$ 3.1 ^{ab}	284.6 $\pm$ 29 ^a	250.3 $\pm$ 29 ^{ab}
Gln	454.0 $\pm$ 56 ^{bc}	472.9 $\pm$ 104 ^{bc}	648.1 $\pm$ 117 ^a	399.4 $\pm$ 45 ^{cd}	528.9 $\pm$ 79 ^b	324.4 $\pm$ 25 ^d
Pro	166.4 $\pm$ 31 ^a	154.2 ^a	188.9 $\pm$ 33 ^a	156.1 $\pm$ 0.1 ^a	199.8 $\pm$ 20 ^a	195.8 $\pm$ 50 ^a
Gly	970.2 $\pm$ 488 ^{bc}	1378.6 $\pm$ 474 ^{ab}	1322.8 $\pm$ 465 ^{ab}	1797.8 $\pm$ 504 ^a	410.0 $\pm$ 56 ^d	492.0 $\pm$ 57 ^{cd}
Ala	417.7 $\pm$ 57 ^b	511.6 $\pm$ 91 ^{ab}	590.9 $\pm$ 172 ^a	601.0 $\pm$ 70 ^a	494.1 $\pm$ 44 ^{ab}	582.0 $\pm$ 165 ^a
Cit	13.6 ^b	42.8 $\pm$ 14 ^b	ND	43.5 $\pm$ 9 ^b	75.7 $\pm$ 12 ^a	92.5 $\pm$ 17 ^a
Val	123.0 $\pm$ 37 ^a	121.4 $\pm$ 21 ^a	112.8 $\pm$ 26 ^a	123.2 $\pm$ 15 ^a	138.9 $\pm$ 11 ^a	145.1 $\pm$ 22 ^a
Cys	72.2 $\pm$ 17 ^a	60.0 $\pm$ 37 ^a	21.4 $\pm$ 22 ^b	53.7 $\pm$ 30 ^{ab}	60.1 $\pm$ 6 ^a	62.2 $\pm$ 29 ^a
Met	64.7 $\pm$ 15 ^{ab}	85.6 $\pm$ 26 ^a	58.2 $\pm$ 12 ^{bc}	77.2 $\pm$ 10 ^{ab}	40.4 $\pm$ 5 ^c	71.1 $\pm$ 20 ^{ab}
Ile	68.2 $\pm$ 21 ^{ab}	54.8 $\pm$ 27 ^b	48.5 $\pm$ 10 ^b	63.0 $\pm$ 7 ^{ab}	61.8 $\pm$ 5 ^{ab}	76.8 $\pm$ 10 ^a
Leu	91.1 $\pm$ 28 ^{bc}	75.9 $\pm$ 21 ^c	65.0 $\pm$ 30 ^c	66.8 $\pm$ 13 ^c	110.3 $\pm$ 8 ^{ab}	133.6 $\pm$ 23 ^a
Tyr	48.8 $\pm$ 8 ^c	52.4 $\pm$ 12 ^c	55.4 $\pm$ 16 ^c	59.6 $\pm$ 7 ^{bc}	75.3 $\pm$ 10 ^{ab}	92.4 $\pm$ 21 ^a
Phe	66.8 $\pm$ 14 ^a	71.0 $\pm$ 8 ^a	69.7 $\pm$ 25 ^a	79.3 $\pm$ 4 ^a	61.9 $\pm$ 5 ^a	74.8 $\pm$ 11 ^a
Trp	33.0 $\pm$ 7 ^b	73.8 $\pm$ 12 ^a	25.8 $\pm$ 7 ^b	72.0 $\pm$ 12 ^a	28.3 $\pm$ 6 ^b	79.6 $\pm$ 30 ^a
Orn	39.6 $\pm$ 4 ^b	57.6 $\pm$ 14 ^b	53.9 $\pm$ 14 ^b	103.9 $\pm$ 67 ^b	65.2 $\pm$ 9 ^b	183.1 $\pm$ 91 ^a
Lys	276.1 $\pm$ 67 ^b	259.0 $\pm$ 94 ^b	244.7 $\pm$ 72 ^{bc}	158.9 $\pm$ 73 ^c	390.0 $\pm$ 54 ^a	405.7 $\pm$ 61 ^a
His	24.2 $\pm$ 5 ^c	30.3 $\pm$ 9 ^a	23.0 $\pm$ 13 ^a	29.1 $\pm$ 4 ^a	28.4 $\pm$ 10 ^a	24.1 $\pm$ 9 ^a
Arg	63.4 $\pm$ 18 ^b	59.6 $\pm$ 25 ^b	73.4 $\pm$ 31 ^b	45.2 $\pm$ 24 ^b	144.6 $\pm$ 27 ^a	135.3 $\pm$ 41 ^a

Values, mean $\pm$ SD, (nmol/mL plasma) without a common superscript within a row differ by $p \leq 0.05$.

of a given experimental period, however, concentrations of both of these amino acids were higher ($p \leq 0.05$) in the groups of the second period relative to those in the first time period. Due to these ambiguities, there may be no physiological basis for a difference in plasma amino acid concentrations due to diet for cys, orn, asp, trp, glu, gln, ala, met and ile.

Threonine, cit, leu, tyr, lys, asn and arg concentrations were clearly depressed in TPN-fed animals relative to the lactalbumin-fed controls (Table 15). Serine and gly concentrations were clearly elevated in the TPN-fed animals.

The key amino acids and metabolites for urea synthesis are: (1) cit, orn and arg due to their role as urea cycle intermediates, (2) lys due to its competition for the γ -carrier in peripheral tissues and possible competition with arg for arginase, and (3) gln due to its role in the gut to provide urea cycle intermediates and/or arg precursors. Glutamine did not appear altered by TPN administration in this protocol; however, spontaneous hydrolysis of the amide N was not controlled during amino acid analysis, thus, the data for gln and glu may not be accurate. Citrulline, orn and arg were all depressed in TPN administered animals relative to the controls. Citrulline was not well detected for the TPN groups, possibly indicating very low concentrations. Ornithine and arg were also depressed in

TPN administered animals, but it should be noted that there was a general trend toward increasing levels of both of these compounds as the percent of arg in the diet increases. Plasma lys, which is an antagonist to arg appeared to decrease with increasing concentrations of arg in the diet.

These data indicate that 4.13% arg in a TPN formula should be sufficient to provide adequate dietary arg under the conditions of a 250 g rat adapted to a lactalbumin diet consisting of 290 mg N, 6.5% arg and 60.2 Kcal/day. The data indicate adequacy in terms of orotate, urinary urea nitrogen, total nitrogen and creatinine excretion.

Assessing Urea Cycle Enzyme Activities Under TPN

A total of eighteen rats were catheterized and placed on TPN. Twelve rats served as control and were fed supplemented lactalbumin diet. All TPN animals were allowed a recovery period, and then were administered an -Arg diet. At the end of the -Arg period, half of the TPN animals were withdrawn from the study for urea cycle enzyme activity determination and a blood flow measurement. At this time, half of the control animals were also withdrawn for the same determinations and measurements and were given the designation Control I. At the end of the 4.5% arg period, the remaining TPN and control animals were withdrawn for the enzyme determinations and blood flow measurement; the

control animals were designated as Control II. Due to time constraints involving the collaborators in this study, the rats were allowed a 2-day recovery period from surgery, a 4-day -Arg period, then a 6-day +Arg period of 4.5% arg.

Table 16 lists the body and organ weights for the groups in this experiment. Weight gain per day was lower ($p \leq 0.05$) in the -Arg group relative to all other diets. The -Arg diet was intravenously administered at the same rate as the 4.5% arg group and was isonitrogenous and isocaloric to the +Arg TPN diet and control diet. Thus, the lower weight gain by the -Arg group indicates that the lack of arginine and not a decrease in food intake is responsible for this effect. Heart and lung weights were not statistically different between dietary groups. The liver was heavier ($p \leq 0.05$) in the -Arg TPN group relative to the Control II group. Duncan's analysis of mean liver weight resulted in an overlapping of nonsignificance between the -Arg, +Arg and Control I groups and between the +Arg, Control I and Control II groups. Thus, the only clear difference in liver weight was between the -Arg group and the Control II group. Kidney weight was not different between groups examined at the same time. Although the kidneys of TPN animals were slightly heavier than their orally-fed counterparts, this increase in weight was not statistically greater. Spleen weight was heavier in the TPN groups relative to the control groups; the 4.5% arg group was heaviest.

Table 16. Body Weight Gain and Organ Weights for Rats Administered 0% Arg or 4.5% Arg by TPN and Their Respective Controls.

	0% Arg	4.5% Arg	Control I	Control II
Body Weight Gain (g/d)				
	0.82 ± 1.8^b	2.88 ± 1.4^a	3.34 ± 0.4^a	3.44 ± 0.2^a
Organ Weight (g/100 g BW)				
Liver	3.61 ± 0.4^a	$3.39 \pm 0.3^{a,b}$	$3.17 \pm 0.2^{a,b}$	2.96 ± 0.2^b
Kidney	0.76 ± 0.03^a	$0.68 \pm 0.01^{b,c}$	$0.72 \pm 0.03^{a,b}$	0.67 ± 0.01^c
Spleen	$0.22 \pm 0.02^{a,b}$	0.24 ± 0.05^a	0.18 ± 0.01^c	$0.22 \pm 0.02^{a,b}$
Lung	0.42 ± 0.2^a	0.54 ± 0.06^a	0.45 ± 0.08^a	0.44 ± 0.02^a
Heart	0.37 ± 0.03^a	0.38 ± 0.07^a	0.35 ± 0.05^a	0.36 ± 0.02^a
GI*	$1.77 \pm 0.1^{a,b}$	1.54 ± 0.02^b	2.49 ± 0.8^a	$1.86 \pm 0.2^{a,b}$

*GI from stomach to cecum without mesentery.

Values (means $\pm$ SD) without a common superscript within a row differ by $p < 0.05$.

Gastrointestinal tract weight was less ($p \leq 0.05$) in the 4.5% arg TPN group relative to Control I. Although there was no statistical difference in GI weight between the -Arg group and the two control groups, the trend was toward lighter weight.

There was no statistical difference in orotate excretion during the recovery period between the three diet groups (Figure 31). Upon -Arg administration, there was a 10-fold increase in orotate excretion which remained elevated throughout the -Arg period. Upon 4.5% arg administration (Day 5), urinary orotate excretion decreased within 24-hours to values which were twice control values. Within 48-hours, there was no difference in orotate excretion between the TPN and control group.

As indicated in Figures 32 and 33, both UUN and urinary total nitrogen excretion followed the same pattern. There was no difference in nitrogen excretion between the three dietary groups during the recovery period. Upon -Arg administration, urinary nitrogen excretion increased 1.5- to 2-fold in the TPN groups and remained elevated throughout the -Arg period. With 4.5% arg supplementation, urinary nitrogen declined and by 96-hours (Day 9), there was no difference in either UUN or TN excretion between the TPN and control groups.

After the recovery period, there was no statistical difference in creatinine excretion between the three dietary

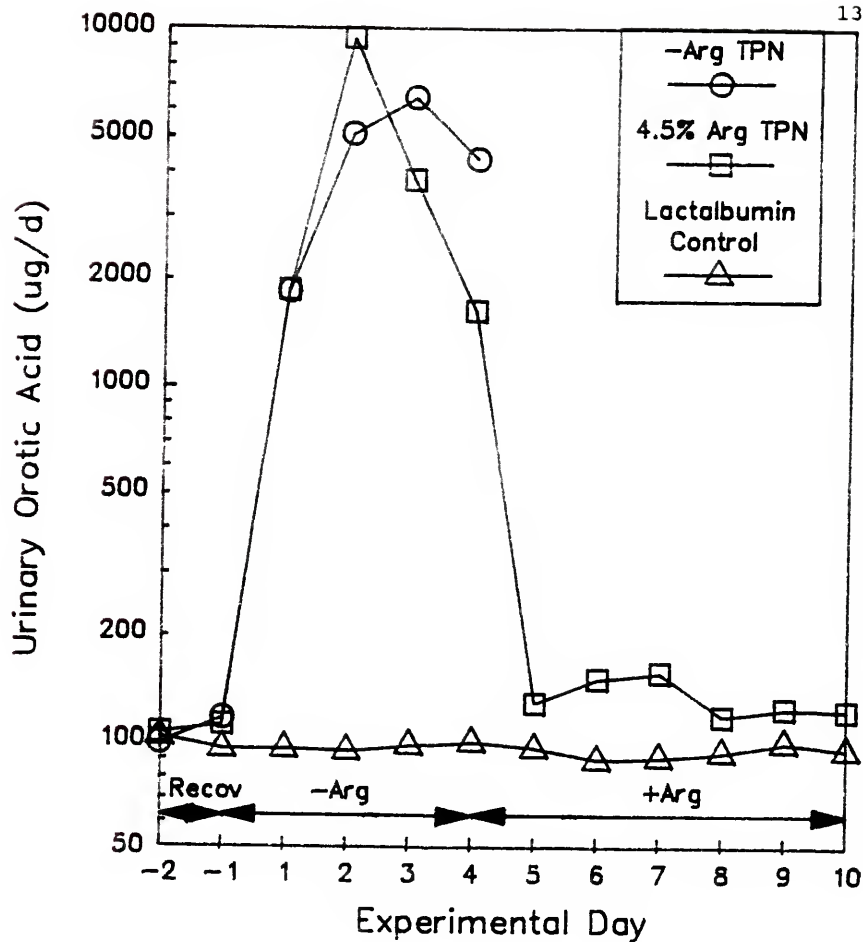


Figure 31. Average Daily Urinary Orotic Acid Excretion of Rats Administered Either 0% Arginine or 4.5% Arginine and Controls.

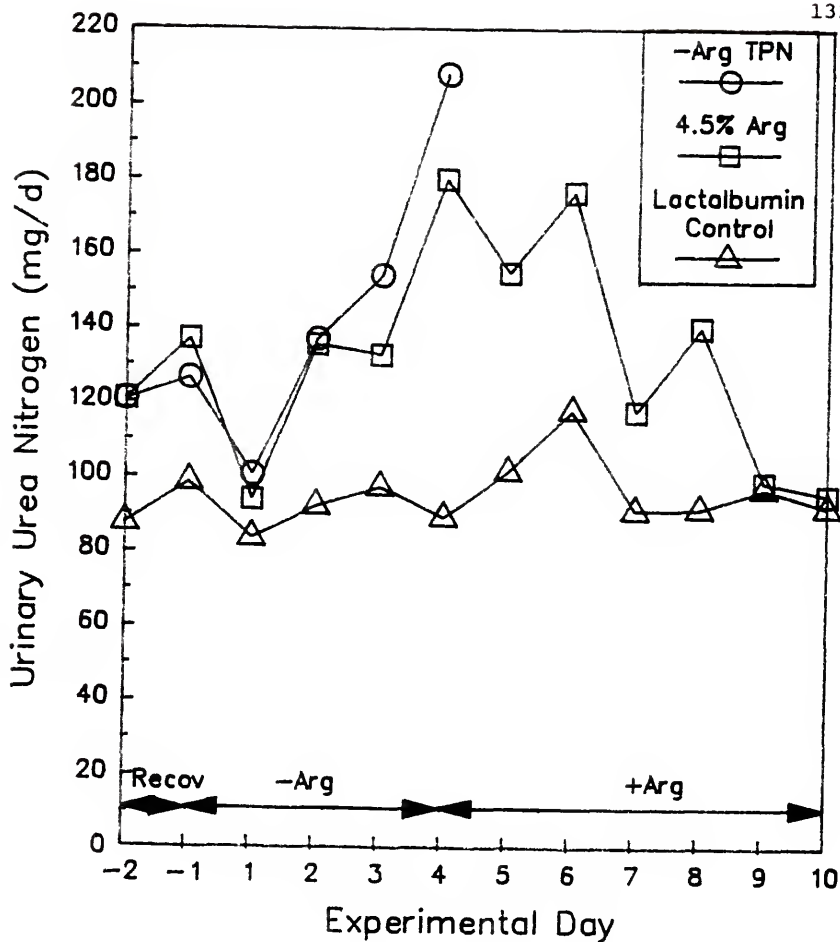


Figure 32. Average Daily Urinary Urea Nitrogen Excretion of Rats Administered Either 0% Arginine or 4.5% Arginine and Controls.

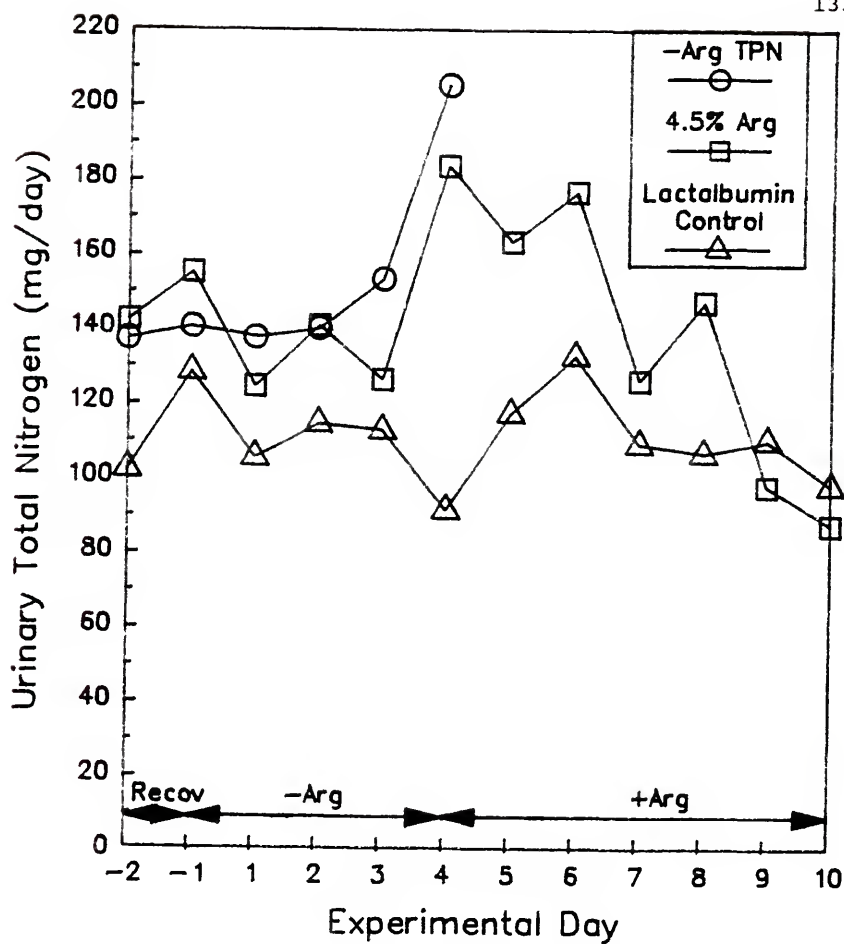


Figure 33. Average Daily Urinary Total Nitrogen Excretion of Rats Administered Either 0% Arginine or 4.5% Arginine and Controls.

groups, except on Day 6 (Figure 34). Individual animal urinary creatinine excretion CV varied between 6.5% and 16.8% which is within normal variations. Thus, it was assumed that urine samples were adequately collected.

Liver CPS activity was elevated in the -Arg group and was depressed in the 4.5% arg ($p \leq 0.05$) (Table 17). Liver AS was elevated in the -Arg group relative to the 4.5% arg group. Duncan's analysis indicated no significant differences for other group comparisons. Thus, the real difference in liver AS activity is due to the level of arginine supplementation in the TPN formulation. Likewise, there was no difference in liver A activity between the -Arg group and the two control groups, but liver A activity in the 4.5% arg group was depressed ($p \leq 0.05$). Arginine is thought to participate in repression of urea cycle enzyme synthesis; this may account for the decreased levels of urea cycle enzyme activities in the liver for the 4.5% arg group. The increase in CPS activity in the -Arg group indicates that the insufficient amount of free arginine resulted in CPS enzyme production. There was no statistical difference in kidney AS activity between the four groups. Kidney A activity was elevated in the 4.5% arg group relative to all other diets.

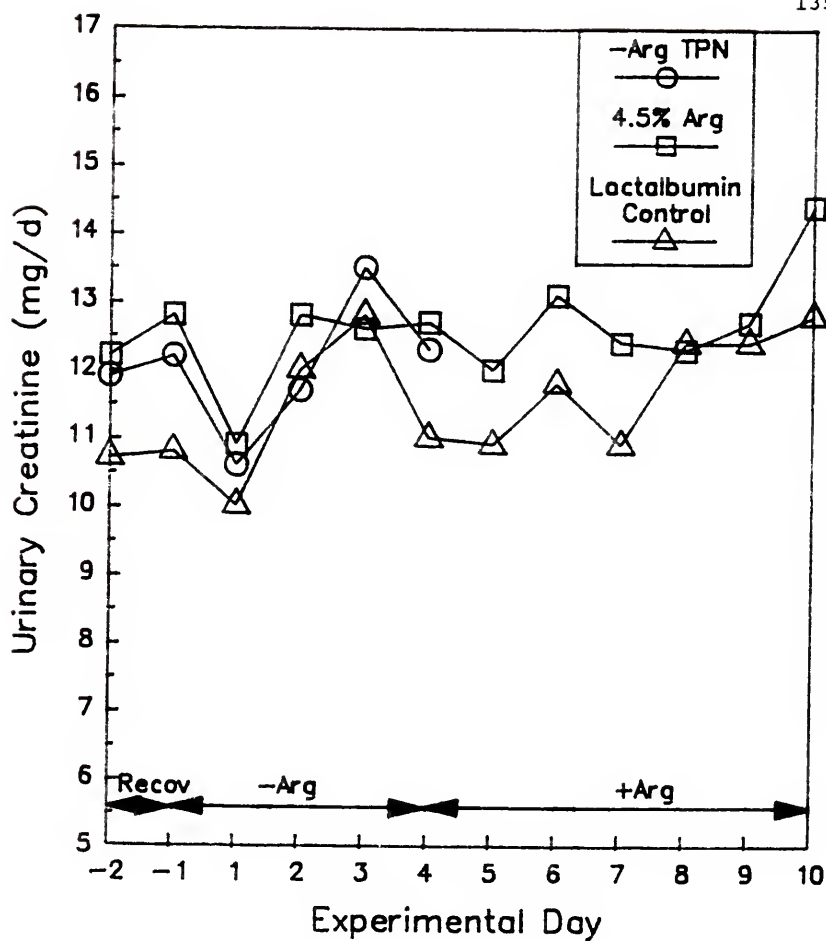


Figure 34. Average Daily urinary Creatinine Excretion of Rats Administered Either 0% Arginine or 4.5% Arginine and Controls.

Table 17. Urea Cycle Enzyme Activities for Rats Administered 0% Arginine or 4.5% Arginine by TPN and Their Respective Controls.

(n)	0% Arg (4)	4.5% Arg (4)	Control I (6)	Control II (6)
Liver Enzyme Activity*				
CPS ($\times 10^5$)	8.74 $\pm$ 7.0 ^a	2.51 $\pm$ 3.3 ^c	3.20 $\pm$ 1.4 ^b	3.31 $\pm$ 1.4 ^b
AS	72.39 $\pm$ 39.0 ^a	31.87 $\pm$ 8.4 ^b	50.44 $\pm$ 31.3 ^{a,b}	47.46 $\pm$ 12.5 ^{a,b}
A ($\times 10^3$)	11.95 $\pm$ 2.6 ^a	7.48 $\pm$ 2.2 ^b	10.82 $\pm$ 3.4 ^a	10.46 $\pm$ 1.5 ^a
Kidney Enzyme Activity*				
AS	26.29 $\pm$ 18.8 ^{a,b}	34.96 $\pm$ 3.2 ^a	22.28 $\pm$ 3.9 ^b	30.24 $\pm$ 15.7 ^a
A ($\times 10^2$)	1.63 $\pm$ 0.4 ^b	6.65 $\pm$ 6.4 ^a	1.18 $\pm$ 0.2 ^b	1.03 $\pm$ 0.8 ^b

* $\mu\text{mol product/hr/g tissue}$

CPS = carbamoyl phosphate synthetase

AS = argininosuccinate synthetase

A = arginase

Determination of Arteriovenous Amino Acid Differences

Arteriovenous amino acid differences and urea cycle enzyme activities were determined in one experiment. Blood flow measurements were also attempted. Due to the difficulty in placement of the 30-gauge needle into the tertiary branch of the mesentery vein, blood flow measurements were 50% successful in the -Arg and Control I groups and were unsuccessful in the +Arg and Control II groups. For this reason, only measurements for the -Arg group and the Control I group are reported. It was assumed that blood flow was not altered by supplementation of arginine in the TPN formula and preliminary amino acid flux calculations were made for both TPN and control groups based on the reported values.

Table 18 shows the blood flow measurements for the TPN and control groups. Blood flow in the portal vein was slightly elevated in the TPN group relative to the control group. Reported values of portal blood flow for control group rats range between 2.8 and 3.2 mL/min/100 g BW (Welbourne et al., 1986a, Welbourne et al., 1986b, Buttrose et al., 1987). Renal blood flow was also elevated in the TPN group relative to the control group. Reported values of renal blood flow for control group rats range between 3.74 and 4.0 mL/min/100 g BW (Welbourne et al., 1986a, Welbourne et al., 1986b). Thus, renal and portal blood flow data for

Table 18. Portal and Renal Vein Blood Flow for Rats on TPN or Lactalbumin Control.

Vein (n)	TPN (3) (mL/min/100 g BW)	Control (4)
Portal	4.22 $\pm$ 1.13	3.00 $\pm$ 2.4
1/2 Renal	5.31 $\pm$ 1.8	4.13 $\pm$ 1.1

Values are mean $\pm$ SD.

control groups from this experiment agree with reported values.

The portal vein transports blood from the small intestine to the liver and the hepatic vein transports blood from the liver to the inferior vena cava and then returns to the heart for oxygenation by the lungs. Measurements of amino acid net uptake are determined by arteriovenous differences and reflect the steady-state balance between influx and efflux of that amino acid across the organ (Pardridge, 1977). Positive values indicate a net uptake of an amino acid, whereas, negative values indicate a net release of an amino acid into peripheral circulation. Multiplying the A-V difference by the blood flow rate yields the amino acid flux of that vein. Organ extraction of an amino acid can be determined by dividing the A-V difference by the arterial concentration of that amino acid.

Amino acid concentrations of the four sites sampled were within the concentration range obtained from previous experiments for this research (Table 24, Table 25, Table 26, Table 27). The coefficient of variation for each amino acid within a group ranged from 4.4 to 67.0% with an average of 32%. Determining the A-V difference ($[AA]_{\text{arterial}} - [AA]_{\text{venous}}$) exaggerated the variation within a group. Due to the low number of animals per group and the wide variation of values within a group, definitive differences between groups were difficult to obtain. Thus, only apparent trends in the data

can be concluded. Liver net uptake of thr, ser, glu, gln, val, cys, ile, tyr and lys was greater in the 0% arg TPN group relative to the 4.5% TPN group and controls (Table 19 and Table 20). Liver net uptake of gly was greater in both TPN groups relative to controls, probably due to the increased concentration of gly in the TPN formula relative to the control diet.

Nearly all amino acids were taken up by the kidney to a greater extent in the 0% arg group than the 4.5% arg group or the control group (Table 21). Glycine was taken up by the kidney to a greater degree by both TPN groups relative to controls. Arginine was released by the kidney in all groups. In humans, the kidney is almost the exclusive site of serine synthesis which is thought to be derived from gly uptake. The kidney has the predominate role for gln disposal and in addition, it predominately releases ala and cys (Tizianello et al., 1980). The kidney A-V data obtained in this experiment is in agreement with the established role of the kidney in amino acid metabolism, except for gln and ala.

Amino acid extraction coefficient and flux for gln, cit, orn and arg are listed in Tables 22 and 23, respectively. Liver extraction of gln, cit and orn was greater in the -Arg TPN group relative to the 4.5% arg TPN group and control groups. The extraction of arg by the liver was similar in both TPN groups. Arginine and orn flux

Table 19. Arteriovenous Amino Acid Differences Between the Carotid Artery and Hepatic Vein of Rats Administered a TPN Formula Containing Either 0% Arginine or 4.5% Arginine to Total Amino Acids and Controls.

Amino Acid (n)	0% Arg TPN (4)	4.5% Arg TPN (2)	Control I (5) (nmol/mL plasma)	Control II (7)
Aspartate	8.13 ± 7.1	1.67 ± 3.0	-1.29 ± 16.4	-4.76 ± 9.4
Threonine	56.88 ± 37.3	21.18 ± 3.9	-63.64 ± 166.2	-11.66 ± 76.8
Serine	130.64 ± 105.9	23.75 ± 46.7	-51.02 ± 164.4	-0.78 ± 58.2
Asparagine	11.42 ± 34.4	n.d.	-126.66 ± 204.0	-6.41 ± 27.7
Glutamate	168.27 ± 115.59	96.40 ± 2.8	46.18 ± 129.0	5.68 ± 88.9
Glutamine	153.17 ± 157.01	9.45 ± 31.6	-8.74 ± 151.6	20.16 ± 53.1
Glycine	304.10 ± 240.1 ^a	167.11 ± 11.2 ^{ab}	-75.82 ± 238.4 ^b	-32.28 ± 88.0 ^b
Alanine	65.91 ± 106.4	50.86 ± 33.8	-181.97 ± 465.2	-46.99 ± 114.6
Citrulline	-5.38 ± 9.2	-3.37 ± 1.9	-23.65 ± 24.6	-15.57 ± 22.9
Valine	10.40 ± 15.6	-1.20 ± 0.1	-53.32 ± 81.6	-2.95 ± 21.9
Cystine	8.12 ± 312.0	-4.10 ± 1.8	-18.41 ± 39.3	13.95 ± 18.6
Methionine	14.61 ± 14.1	8.93 ± 1.7	-9.01 ± 29.0	4.25 ± 8.0
Isoleucine	4.19 ± 14.8	3.79 ± 1.1	-24.03 ± 45.9	13.84 ± 57.3
Leucine	15.59 ± 15.8	9.09 ± 1.2	-48.10 ± 96.7	-12.15 ± 24.7
Tyrosine	10.50 ± 8.4	0.49 ± 1.8	-7.04 ± 34.6	-0.82 ± 13.6
Phenylalanine	22.82 ± 12.2	13.09 ± 2.4	-13.83 ± 39.1	-4.54 ± 15.6
Ornithine	7.30 ± 25.9	-0.86 ± 0.8	-17.80 ± 35.5	-13.80 ± 41.1
Lysine	53.38 ± 84.1	26.41 ± 35.5	-26.11 ± 160.4	11.82 ± 84.9
Histidine	9.42 ± 13.2	n.d.	-16.17 ± 25.2	-0.76 ± 7.9
Arginine	7.61 ± 13.2	7.66 ± 1.0	-10.33 ± 66.9	6.24 ± 17.3

Values (mean ± SD) without a common superscript within a row differ by $p < 0.05$.

Table 20. Arteriovenous Amino Acid Differences Between the Carotid Artery and Portal Vein of Rats Administered a TPN Formula Containing Either 0% Arginine or 4.5% Arginine to Total Amino Acids and Controls.

Amino Acid (n)	0% Arg TPN (4)	4.5% Arg TPN (2) (nmol/mL plasma)	Control I (8)	Control II (7)
Aspartate	-0.19 ± 11.4	4.38 ± 2.8	-4.85 ± 23.9	-2.82 ± 8.3
Threonine	13.41 ± 12.4	5.41 ± 11.6	-17.10 ± 51.9	-17.10 ± 51.9
Serine	37.92 ± 36.9 ^a	20.58 ± 0.4 ^a	-54.15 ± 39.0 ^b	-7.48 ± 37.0 ^{a,b}
Asparagine	-96.60 ± 77.5	n.d.	-193.26 ± 103.4	-27.35 ± 69.9
Glutamate	158.47 ± 66.3 ^a	72.71 ± 35.3 ^{a,b}	79.04 ± 92.4 ^{a,b}	23.81 ± 36.8 ^b
Glutamine	153.30 ± 147.2 ^a	27.52 ± 33.3 ^b	77.46 ± 79.8 ^{a,b}	20.52 ± 22.8 ^b
Glycine	47.90 ± 69.6 ^a	17.17 ± 68.6 ^a	-153.30 ± 75.6 ^b	-49.8 ± 71.8 ^{a,b}
Alanine	-144.62 ± 55.8 ^a	-65.92 ± 65.8 ^a	-344.71 ± 103.3 ^b	-102.71 ± 130.4 ^a
Citrulline	-13.40 ± 7.7	-4.22 ± 4.2	-27.88 ± 13.0	-11.42 ± 21.0
Valine	4.02 ± 13.0 ^a	8.97 ± 2.9 ^a	-44.55 ± 22.0 ^b	2.38 ± 19.8 ^a
Cystine	25.81 ± 10.4	5.04 ± 0.5	-7.39 ± 33.3	9.37 ± 11.3
Methionine	2.04 ± 5.8 ^a	-0.14 ± 1.7 ^a	-17.95 ± 15.4 ^b	2.36 ± 10.0 ^a
Isoleucine	4.45 ± 10.5	0.44 ± 1.4	-35.06 ± 31.3	12.39 ± 49.7
Leucine	5.31 ± 8.3 ^a	-1.53 ± 7.1 ^a	-61.72 ± 29.4 ^b	-9.23 ± 22.3 ^a
Tyrosine	0.70 ± 2.5	-1.15 ± 1.7	-12.75 ± 14.6	-5.59 ± 11.3
Phenylalanine	2.45 ± 3.8	2.37 ± 3.8	-16.64 ± 10.2	-8.06 ± 15.5
Ornithine	14.71 ± 18.9 ^a	11.54 ± 23.6 ^a	-24.26 ± 23.6 ^b	10.52 ± 21.9 ^a
Lysine	57.11 ± 27.7 ^a	14.96 ± 86.2 ^a	-109.08 ± 96.7 ^b	-6.06 ± 67.9 ^{a,b}
Histidine	17.27 ± 18.6	n.d.	-14.81 ± 34.9	-0.32 ± 10.2
Arginine	-7.22 ± 10.6	-5.89	-20.56 ± 18.7	-7.95 ± 18.7

Values (mean ± SD) without a common superscript within a row differ by $p < 0.05$.

Table 21. Arteriovenous Amino Acid Differences Between the Carotid Artery and Renal Vein of Rats Administered a TPN Formula Containing Either 0% Arginine or 4.5% Arginine to Total Amino Acids and Controls.

Amino Acid (n)	0% Arg TPN (4)	4.5% Arg TPN (2)	Control I (5) (nmol/mL plasma)	Control II (7)
Aspartate	26.39 ± 7.1	18.56 ± 1.3	5.84 ± 9.4	-3.25 ± 17.5
Threonine	18.53 ± 68.4	3.60 ± 11.2	-3.29 ± 87.6	-25.85 ± 68.2
Serine	-23.15 ± 127.1	-53.60 ± 29.1	-52.61 ± 75.9	-53.60 ± 29.1
Asparagine	35.68 ± 18.7	n.d.	-22.57 ± 66.7	-151.10 ± 268.4
Glutamate	38.43 ± 97.1	17.74 ± 26.0	113.46 ± 105.1	-16.67 ± 94.4
Glutamine	22.91 ± 131.4	-15.95 ± 0.9	6.81 ± 99.4	1.02 ± 47.4
Glycine	70.53 ± 203.7	54.89 ± 90.9	8.92 ± 108.6	0.58 ± 48.0
Alanine	123.71 ± 208.6	7.47 ± 30.5	39.58 ± 173.75	-23.52 ± 62.7
Citrulline	7.67 ± 13.8	6.69 ± 5.2	10.21 ± 8.9	4.22 ± 13.7
Valine	11.56 ± 39.3	14.23 ± 12.0	-7.98 ± 47.5	4.49 ± 17.4
Cysteine	-11.01 ± 23.6	-7.81 ± 0.1	-16.21 ± 20.3	-9.86 ± 17.7
Methionine	2.26 ± 11.5	-0.92 ± 3.8	-2.57 ± 19.3	1.18 ± 8.4
Isoleucine	13.08 ± 29.8	0.69 ± 3.6	-1.32 ± 21.4	17.08 ± 48.2
Leucine	22.80 ± 41.4	0.80 ± 8.6	-3.96 ± 40.7	-5.21 ± 8.2
Tyrosine	1.94 ± 21.1	-6.15 ± 3.1	-3.64 ± 22.0	-10.20 ± 13.3
Phenylalanine	16.76 ± 17.0	8.44 ± 4.1	2.71 ± 17.7	-4.22 ± 9.7
Ornithine	6.05 ± 31.5	9.85 ± 20.9	-0.87 ± 26.0	-10.72 ± 30.5
Lysine	56.58 ± 120.9	63.84 ± 30.1	36.62 ± 65.0	-23.53 ± 57.9
Histidine	-2.41 ± 15.5	n.d.	-0.86 ± 5.6	-6.23 ± 9.9
Arginine	-3.63 ± 15.4	-5.06 ± 1.4	-14.23 ± 33.5	-15.26 ± 5.1

Values (mean ± SD) without a common superscript within a row differ by $p < 0.05$.

Table 22. Extraction Coefficient for Glutamine, Citrulline, Ornithine and Arginine in the Liver and Kidney of Rats Administered Either 0% or 4.5% Arginine TPN and Controls.

	Gln	Cit	Orn	Arg
Liver				
0% Arg	-0.03 $\pm$ 0.2	0.52 $\pm$ 0.6	-0.27 $\pm$ 0.4	0.32 $\pm$ 0.6
4.5% Arg	-0.27 $\pm$ 0.1	0.02 $\pm$ 0.1	-0.11 $\pm$ 0.2	0.39 $\pm$ 0.1
Control I	-0.27 $\pm$ 0.6	0.24 $\pm$ 0.7	-0.11 $\pm$ 0.4	0.07 $\pm$ 0.5
Control II	-0.31 $\pm$ 0.8	0.40 $\pm$ 0.8	-0.50 $\pm$ 0.4	0.60 $\pm$ 0.9
Kidney				
0% Arg	-0.09 $\pm$ 0.4	0.12 $\pm$ 0.7	-0.06 $\pm$ 0.5	-0.32 $\pm$ 0.4
4.5% Arg	-0.24 $\pm$ 0.1	0.35 $\pm$ 0.2	0.08 $\pm$ 0.2	-0.18 $\pm$ 0.1
Control I	0.01 $\pm$ 0.4	0.30 $\pm$ 0.2	0.01 $\pm$ 0.2	-0.07 $\pm$ 0.3
Control II	-0.23 $\pm$ 0.6	0.43 $\pm$ 0.5	-0.33 $\pm$ 0.5	-0.70 $\pm$ 0.3

Values are mean $\pm$ SD.

Table 23. Amino Acid Flux for Glutamine, Citrulline, Ornithine and Arginine in the Portal and Kidney Veins of Rats Administered Either 0% or 4.5% Arginine TPN and Controls.

	Gln	Cit (nmol/min ⁻¹ 100 g BW ⁻¹)	Orn (nmol/min ⁻¹ 100 g BW ⁻¹)	Arg
Portal Vein				
0% Arg	646.92 ± 537.9	-56.55 ± 28.0	62.06 ± 69.2	-30.45 ± 38.8
4.5% Arg	116.13 ± 99.3	-17.81 ± 12.5	48.71 ± 70.5	-24.86 ± 10.9
Control I	232.39 ± 221.7	-83.63 ± 36.0	-72.79 ± 64.6	-61.69 ± 51.2
Control II	61.55 ± 64.1	-34.27 ± 59.1	31.54 ± 60.7	-23.86 ± 51.3
Full Kidney				
0% Arg	243.35 ± 120.8	81.52 ± 126.9	64.22 ± 29.0	-38.55 ± 141.6
4.5% Arg	-169.43 ± 6.7	71.04 ± 39.3	104.62 ± 157.2	-53.75 ± 10.9
Control I	56.27 ± 73.4	84.31 ± 66.0	-7.15 ± 19.2	-117.54 ± 247.4
Control II	-81.12 ± 39.6	34.82 ± 104.8	-88.54 ± 23.6	-126.03 ± 39.2

Values are mean ± SD.

Table 24. Plasma Amino Acid Concentration in the Carotid Artery of Rats Administered a TPN Formula Containing Either 0% Arginine or 4.5% Arginine to Total Amino Acids and Controls.

Amino Acid (n)	0% Arg TPN (4)	4.5% Arg TPN (2) (nmol/mL plasma)	Control I (5)	Control II (7)
Aspartate	27.68 ± 6.5	24.28 ± 4.8	14.57 ± 4.4	11.36 ± 3.3
Threonine	129.36 ± 41.6	87.33 ± 4.8	172.11 ± 13.0	98.89 ± 32.9
Serine	239.85 ± 44.9	257.14 ± 45.2	172.25 ± 17.6	94.06 ± 26.3
Asparagine	35.17 ± 33.7	n.d.	69.12 ± 7.7	24.50 ± 19.1
Glutamate	294.91 ± 64.1	165.52 ± 11.8	229.30 ± 35.1	105.69 ± 33.8
Glutamine	163.67 ± 74.5	40.57 ± 18.0	110.22 ± 43.1	53.65 ± 20.4
Glycine	462.28 ± 48.6	429.61 ± 93.1	206.36 ± 24.7	100.80 ± 32.3
Alanine	245.88 ± 72.6	180.63 ± 30.0	300.90 ± 35.9	124.39 ± 31.6
Citrulline	11.55 ± 4.5	11.24 ± 2.4	16.12 ± 4.5	14.54 ± 5.6
Valine	78.93 ± 20.9	47.56 ± 7.0	55.03 ± 20.5	24.34 ± 13.1
Cystine	50.20 ± 11.5	28.08 ± 7.7	43.18 ± 12.5	29.12 ± 9.2
Methionine	40.27 ± 9.5	26.85 ± 0.4	21.77 ± 4.4	16.25 ± 3.2
Isoleucine	40.61 ± 14.6	16.59 ± 0.4	24.24 ± 4.5	23.98 ± 24.3
Leucine	71.14 ± 24.28	28.58 ± 2.8	50.50 ± 9.3	19.81 ± 5.4
Tyrosine	31.20 ± 12.6	16.27 ± 2.3	31.41 ± 5.6	18.24 ± 5.0
Phenylalanine	43.05 ± 9.0	31.84 ± 4.0	27.39 ± 3.4	15.16 ± 5.0
Ornithine	40.31 ± 15.9	37.87 ± 12.0	58.34 ± 14.6	36.49 ± 19.2
Lysine	202.75 ± 75.3	96.62 ± 7.8	202.05 ± 38.3	111.17 ± 32.8
Histidine	34.94 ± 6.2	n.d.	13.42 ± 7.0	5.20 ± 3.4
Arginine	16.67 ± 8.0	15.83 ± 7.5	44.86 ± 20.2	10.22 ± 6.2

Values are mean ± SD.

Table 25. Plasma Amino Acid Concentration in the Hepatic Vein of Rats Administered a TPN Formula Containing Either 0% Arginine or 4.5% Arginine to Total Amino Acids and Controls.

Amino Acid (n)	0% Arg TPN (4)	4.5% Arg TPN (2) (nmol/mL plasma)	Control I (5)	Control II (7)
Aspartate	23.62 ± 8.4	23.39 ± 7.6	15.21 ± 3.5	10.05 ± 9.8
Threonine	100.92 ± 35.3	76.74 ± 3.4	203.93 ± 75.1	81.33 ± 44.4
Serine	174.53 ± 42.6	245.26 ± 61.7	197.76 ± 78.3	123.24 ± 82.6
Asparagine	30.59 ± 16.4	n.d.	105.28 ± 67.2	20.10 ± 16.8
Glutamate	210.78 ± 30.3	117.32 ± 10.8	206.21 ± 63.6	122.47 ± 72.7
Glutamine	87.08 ± 22.2	35.85 ± 6.8	114.59 ± 75.2	47.90 ± 32.6
Glycine	310.23 ± 74.7	346.06 ± 97.1	244.27 ± 109.5	185.25 ± 127.5
Alanine	212.92 ± 83.4	155.20 ± 42.0	391.88 ± 218.5	141.95 ± 86.6
Citrulline	14.24 ± 7.8	12.92 ± 3.1	27.95 ± 11.0	15.55 ± 10.0
Valine	73.73 ± 17.6	48.16 ± 7.0	81.68 ± 50.2	41.58 ± 26.7
Cystine	46.13 ± 8.5	30.13 ± 8.3	53.39 ± 28.1	27.29 ± 16.0
Methionine	32.97 ± 5.9	22.39 ± 1.0	26.27 ± 11.8	18.98 ± 11.4
Isoleucine	38.51 ± 12.7	14.70 ± 0.8	36.26 ± 21.6	20.10 ± 15.4
Leucine	63.34 ± 20.8	24.03 ± 3.2	74.55 ± 48.2	33.25 ± 24.8
Tyrosine	25.95 ± 10.2	16.03 ± 1.6	34.93 ± 13.0	17.07 ± 10.6
Phenylalanine	31.64 ± 8.8	25.29 ± 3.2	34.30 ± 16.8	20.39 ± 11.7
Ornithine	36.66 ± 8.8	38.30 ± 11.7	66.94 ± 28.4	33.47 ± 19.6
Lysine	176.06 ± 48.7	83.41 ± 20.3	215.11 ± 65.2	106.62 ± 66.8
Histidine	30.23 ± 5.6	n.d.	21.50 ± 14.4	11.28 ± 9.8
Arginine	13.05 ± 5.1	12.00 ± 7.8	50.02 ± 36.0	9.47 ± 6.2

Values are mean ± SD.

Table 26. Plasma Amino Acid Concentration in the Portal Vein of Rats Administered a TPN Formula Containing Either 0% Arginine or 4.5% Arginine to Total Amino Acids and Controls.

Amino Acid (n)	0% Arg TPN (4)	4.5% Arg TPN (2) (nmol/mL plasma)	Control I (8)	Control II (7)
Aspartate	25.88 $\pm$ 6.1	22.09 $\pm$ 5.8	17.40 $\pm$ 8.9	12.29 $\pm$ 4.3
Threonine	109.98 $\pm$ 40.0	84.63 $\pm$ 8.8	189.44 $\pm$ 47.1	95.04 $\pm$ 29.6
Serine	229.54 $\pm$ 30.7	246.86 $\pm$ 45.0	185.49 $\pm$ 47.3	89.96 $\pm$ 28.4
Asparagine	47.60 $\pm$ 27.6	n.d.	160.25 $\pm$ 53.9	45.74 $\pm$ 33.5
Glutamate	186.84 $\pm$ 49.1	129.17 $\pm$ 24.3	176.55 $\pm$ 56.4	87.72 $\pm$ 30.2
Glutamine	66.95 $\pm$ 21.8	26.81 $\pm$ 23.3	65.72 $\pm$ 22.6	37.00 $\pm$ 13.3
Glycine	432.56 $\pm$ 26.2	421.02 $\pm$ 117.4	268.08 $\pm$ 66.9	114.79 $\pm$ 39.9
Alanine	283.32 $\pm$ 71.1	213.59 $\pm$ 53.2	437.40 $\pm$ 119.8	167.82 $\pm$ 55.0
Citrulline	16.62 $\pm$ 6.9	13.35 $\pm$ 3.9	29.54 $\pm$ 7.6	20.61 $\pm$ 5.0
Valine	65.64 $\pm$ 20.9	43.07 $\pm$ 6.0	66.89 $\pm$ 17.6	28.26 $\pm$ 4.8
Cystine	33.38 $\pm$ 7.9	25.56 $\pm$ 7.5	38.57 $\pm$ 12.6	21.69 $\pm$ 8.9
Methionine	35.15 $\pm$ 7.2	26.93 $\pm$ 0.2	29.32 $\pm$ 8.9	15.88 $\pm$ 1.8
Isoleucine	31.05 $\pm$ 15.8	16.37 $\pm$ 0.1	41.05 $\pm$ 13.9	15.83 $\pm$ 5.3
Leucine	55.44 $\pm$ 25.6	29.34 $\pm$ 5.3	78.06 $\pm$ 17.8	25.84 $\pm$ 6.5
Tyrosine	26.18 $\pm$ 13.0	16.85 $\pm$ 2.9	37.27 $\pm$ 9.3	19.38 $\pm$ 5.6
Phenylalanine	38.10 $\pm$ 9.9	30.65 $\pm$ 5.4	30.02 $\pm$ 14.6	18.99 $\pm$ 4.7
Ornithine	32.67 $\pm$ 16.8	32.10 $\pm$ 3.6	55.67 $\pm$ 30.7	25.06 $\pm$ 18.2
Lysine	145.84 $\pm$ 71.1	89.14 $\pm$ 22.7	207.76 $\pm$ 111.4	97.30 $\pm$ 43.3
Histidine	15.99 $\pm$ 5.2	n.d.	18.59 $\pm$ 11.8	4.71 $\pm$ 3.2
Arginine	17.90 $\pm$ 2.5	13.14 $\pm$ 7.8	44.79 $\pm$ 29.9	16.63 $\pm$ 8.0

Values are mean $\pm$ SD.

Table 27. Plasma Amino Acid Concentration in the Renal Vein of Rats Administered a TPN Formula Containing Either 0% Arginine or 4.5% Arginine to Total Amino Acids and Controls.

Amino Acid (n)	0% Arg TPN (4)	4.5% Arg TPN (2) (nmol/mL plasma)	Control I (5)	Control II (7)
Aspartate	13.40 $\pm$ 8.7	14.99 $\pm$ 5.2	11.65 $\pm$ 3.6	13.05 $\pm$ 8.7
Threonine	102.52 $\pm$ 25.2	85.53 $\pm$ 8.7	173.76 $\pm$ 41.3	105.00 $\pm$ 30.9
Serine	220.01 $\pm$ 58.6	283.94 $\pm$ 34.9	215.85 $\pm$ 50.9	116.53 $\pm$ 31.5
Asparagine	24.70 $\pm$ 14.8	n.d.	80.40 $\pm$ 35.4	69.80 $\pm$ 38.4
Glutamate	236.50 $\pm$ 58.6	156.66 $\pm$ 21.0	172.57 $\pm$ 32.6	118.03 $\pm$ 44.0
Glutamine	130.34 $\pm$ 30.4	48.55 $\pm$ 18.3	106.82 $\pm$ 49.1	47.95 $\pm$ 27.0
Glycine	377.04 $\pm$ 113.2	402.16 $\pm$ 125.2	201.90 $\pm$ 51.1	83.88 $\pm$ 42.6
Alanine	161.45 $\pm$ 47.6	176.89 $\pm$ 40.8	281.11 $\pm$ 76.0	112.92 $\pm$ 55.5
Citrulline	6.55 $\pm$ 3.0	7.90 $\pm$ 4.3	11.02 $\pm$ 3.8	11.19 $\pm$ 7.5
Valine	65.32 $\pm$ 22.6	40.44 $\pm$ 2.7	59.02 $\pm$ 24.9	24.08 $\pm$ 10.8
Cystine	48.60 $\pm$ 17.5	31.99 $\pm$ 7.8	51.29 $\pm$ 13.8	28.60 $\pm$ 12.0
Methionine	35.48 $\pm$ 13.6	27.32 $\pm$ 1.8	23.06 $\pm$ 6.8	14.30 $\pm$ 6.4
Isoleucine	30.59 $\pm$ 15.8	16.24 $\pm$ 1.7	24.91 $\pm$ 10.6	12.96 $\pm$ 5.9
Leucine	53.03 $\pm$ 24.6	28.18 $\pm$ 0.2	52.48 $\pm$ 22.5	20.64 $\pm$ 8.8
Tyrosine	26.40 $\pm$ 11.6	19.35 $\pm$ 1.2	33.23 $\pm$ 7.7	22.20 $\pm$ 5.7
Phenylalanine	30.14 $\pm$ 7.3	27.62 $\pm$ 2.6	26.03 $\pm$ 6.9	17.96 $\pm$ 4.6
Ornithine	32.13 $\pm$ 17.8	32.94 $\pm$ 4.6	58.78 $\pm$ 20.8	38.15 $\pm$ 10.6
Lysine	147.60 $\pm$ 27.9	64.70 $\pm$ 2.9	183.74 $\pm$ 46.4	119.86 $\pm$ 24.2
Histidine	29.10 $\pm$ 1.5	n.d.	13.23 $\pm$ 8.7	7.37 $\pm$ 5.0
Arginine	13.36 $\pm$ 9.3	18.36 $\pm$ 8.0	51.97 $\pm$ 29.0	19.98 $\pm$ 5.6

Values are mean $\pm$ SD.

from the portal vein were similar for all groups. Influx of gln from the portal vein was greatest in the -Arg group. Efflux of cit from the portal vein was depressed in the TPN groups relative to their respective controls. Without a hepatic blood flow value, relative uptake by the liver of these key amino acids can not be determined.

Kidney extraction of cit was depressed in the -Arg group relative to the other groups. Except for the control II group, cit influx was similar in all groups. Arginine efflux from the kidney was depressed in the TPN groups relative to the controls; however, average arg efflux was greater in the 4.5% arg TPN group relative to the 0% arg TPN group. There was extreme variation in results obtained for the arg A-V difference in the -Arg group, thus only an overall trend can be concluded for arg release by the two TPN groups.

In conclusion, arginine extraction by the liver does not appear to be altered by the concentration of arg in the TPN formula. Due to the discrepancy in extraction values obtained for arg by the liver of control animals, it is not possible to compare the arg extraction capacity of the liver of TPN and orally-fed rats. Although there is less release of arg by the kidney in the 4.5% arg group, the increased arg efflux coupled with equivalent arg extraction by the liver would allow increased arg availability for peripheral

use in the 4.5% arg group relative to the 0% arg group. Due to the low number of animals per group and the large variation in values, more data are necessary to arrive at a definitive conclusion regarding the relative arg supply to peripheral circulation provided by the kidney.

SUMMARY AND CONCLUSIONS

A rat model was developed for the study of arginine metabolism during TPN administration and established the concentration of arg required as a percentage of total amino acids in the TPN formula. In addition, this research examined the effect of an arg-devoid and adequately arg-supplemented TPN formula on urea cycle enzyme activities of the liver and kidney. Arteriovenous amino acid differences across the liver and kidney were also quantified.

Rats (250 ± 25 g, Sprague-Dawley) were adapted to a lactalbumin diet which was isonitrogenous to the subsequent TPN formula and was supplemented with arg to obtain an arg/total protein ratio of 0.065. The TPN formula provided 290 mg N and 60 Kcal per day along with micronutrients at levels previously established to promote normal healthy growth in rats. Rats were allowed a 3-day post-operative recovery period to minimize the nitrogen response from surgery and subsequent dietary arg depletion. A 4-day arg-devoid TPN diet was adequate to deplete the arg stores in the rats as indicated by a 10-fold increase in urinary orotic acid excretion and a 5-fold increase in urinary citric acid excretion. A 5-day adequately arg-supplemented TPN administration was sufficient to return urinary metabolite excretion levels to normal values. Metabolic

stability of the rat was critical for this research since stress induces a nitrogen response in which there is increased amino acid flux to the liver. To establish a minimal arg requirement in TPN for rats, individual animal variation in nitrogen response to stress must be minimized since arg requirements are influenced by the total nitrogen flux to the liver.

Feeding graded levels of arg after the 4-day arg-depletion period resulted in a strong correlation between increasing arg content in the TPN formula and decreasing orotic acid excretion values. A TPN formula supplemented to 4.13% arg/TAA was adequate to reduce daily urinary orotic acid excretion to 100 μ g or less, a level consistent with that reported for healthy, well-nourished orally-fed rats of this body weight. This amount of arg in the TPN formula was also adequate to produce normal values of urinary nitrogen. It was also lower than that found for orally-fed rats in this study as well as other studies. Rats fed a lactalbumin-based semipurified diet required supplementation to 6.5% arg of total protein to minimize urinary orotic acid and urinary urea nitrogen excretion. Milner and Visek (1978) determined that a supplementation to 10.44% arg to total amino acids was required to maintain minimum levels of orotic acid excretion for rats fed a purified diet. Thus, data from this research indicate that under conditions of metabolic stability the requirement of arg in TPN is lower than that for orally-fed rats. Other investigators have

studied arg need in TPN by analyzing immune function. Barbul et al. (1984) determined an optimal arg content in TPN for 275-350 g, Sprague-Dawley rats based on the immunological response of thymic weight, total number of lymphocytes/thymus and mitogen-induced thymocyte blastogenesis from formulas supplemented to 3.6, 9.5 and 18.1% arg/TAA. Their data showed a level of arg supplementation of 18.1% of total amino acids provided a maximal response. However, maximal immunological response is not generally regarded as a parameter for establishing nutrient adequacy or requirements. The data from this dissertation research establishes adequacy of arg based on changes in metabolic parameters, a protocol that is widely utilized to estimate nutrient requirements.

Glutamine, a precursor for arg biosynthesis, was studied for its potential to substitute for arg in TPN. A formula containing gln at a level 5.4% of the total amino acids (or 0.13% of the diet) was evaluated. This concentration represented isonitrogenous replacement of the 3.25% arg/TAA formula. However, this replacement level was inadequate to prevent orotic aciduria. From data presented by Windmueller and Spaeth (1978, 1981), it can be calculated that of the quantity of gln normally metabolized by the gut, approximately 3% could subsequently be synthesized into arg by the kidneys. Thus, gln replacement for arg in a TPN formula would need to be approximately 30 times that of arg normally contained in a TPN amino acid mixture. Recent

studies regarding the use of gln supplementation in TPN for maintenance of gut function include gln at levels of 1 and 2% of the total formula (Grant and Snyder, 1988). These levels of supplementation are equivalent to 23.5% and 47.0% gln/TAA, respectively. Thus, a significantly greater quantity of gln than those levels used in this research may be necessary to produce a beneficial response relative to arg metabolism.

An arg-devoid formula resulted in elevated liver carbamoyl phosphate synthetase activities relative to control rats and rats on a TPN formula supplemented to 4.5% arg. Liver argininosuccinate synthetase and arginase activities from animals administered an arg-devoid TPN formula were not different from control rats. Supplementing a TPN formula to 4.5% arg (as a percent of total amino acids) resulted in depressed liver carbamoyl phosphate synthetase and arginase activities relative to rats administered an -Arg TPN formula and controls. Liver argininosuccinate synthetase activity in the 4.5% arg/TAA group was depressed relative to the arg-devoid TPN group. Kidney argininosuccinate synthetase activity was not affected by an the supplementation or deletion of arg in the TPN formulation. Supplementing the TPN formula to 4.5% arg/TAA resulted in elevated kidney arginase activity. Recent literature reports on the control of urea cycle enzyme synthesis indicates that arg plays a repressive role in RNA transcription in hepatocytes. This evidence seems to

explain the alterations in liver urea cycle enzyme activities measured in this research. Liver urea cycle enzyme activities do not appear to be altered by the practice of TPN but, rather are responsive to the level of arg in the TPN formula.

In conclusion, this research established by regression analysis that a supplementation level of only 4.1% arg/TAA in a TPN formulation is adequate for 250 ± 25 g Sprague-Dawley adult rats. This level is actually lower than that required for orally-fed rats. There is no conclusive evidence in the literature that adult humans have any need for arg in TPN. There have been only four case reports of arg-responsive hyperammonemia in infants given synthetic amino acid solutions. Of all amino acid solutions used for TPN, approximately 99% are administered to adults and the remainder to infants and children (Dr. T.G. Baumgartner, personal communication). These data, coupled with the lack of evidence for increased arg needs during TPN in adult humans, do not justify the high arg content in amino acid mixtures for TPN. Both young animals and infant humans however, may have a markedly different requirement. This remains to be determined.

APPENDIX I DIETARY COMPOSITIONS

This appendix contains the dietary compositions for the experiments conducted for this research. The first diets listed are those used as a result of experiments to develop an appropriate model. The latter diets listed are those used in the development of the model.

Supplemented Lactalbumin Control Diet:

<u>Compound</u>	<u>g/Kg diet</u>
Lactalbumin (12.4% N)	145
L-Arg-HCl	*
Corn Oil	100
AIN 76A Mineral Mix	35
AIN 76A Vitamin Mix	10
Choline Bitartrate	2
Non-nutritive Cellulose Fiber	10
Sucrose	233
Corn Starch	465

<u>Nutrient Density:</u>	(per Kg diet)
Non-N Kcal	3692
g N	18
Non-N Kcal/g N	205.1

* Arginine content is 2.8% of the total protein according to amino acid analysis. Arginine-HCl was added at a rate of 4.4g/100 lactalbumin to achieve a supplementation of 6.5g arg/100g lactalbumin protein. Lactalbumin was supplemented first and then added at the level indicated in the formulation.

AIN 76A Mineral Mix provisions when used at 3.5% of diet

<u>Element</u> ¹	<u>3.5% in diet provides</u>
Calcium	5200.0
Phosphorus	4000.0
Sodium	1020.0
Potassium	3600.0
Magnesium	500.0
Manganese	54.0
Iron	35.0
Copper	6.0
Zinc	30.0
Iodine	0.2
Selenium	0.1
Chromium	2.0
Chloride	1560.0
Sulfate	1000.0

¹Expressed as mg/kg diet

AIN 76A Vitamin Mix provisions when used at 1% of diet

<u>Vitamin</u> ¹	<u>1% in diet provides</u>
Thiamin-HCl, mg/kg	6.0
Riboflavin, mg/kg	6.0
Pyridoxine-HCl, mg/kg	7.0
Nicotinic acid, mg/kg	30.0
Calcium pantothenate, mg/kg	16.0
Folic acid, mg/kg	2.0
Biotin, mg/kg	0.2
Cyanocobalamin, µg/kg	10.0
Vitamin A, IU/kg	4000.0
Vitamin D ₃ , IU/kg	1000.0
Vitamin E ² , IU/kg	50.0
Vitamin K ³ , µg/kg	50.0

¹ expressed per kg diet² [d]-α-tocopherol equivalent, 35 mg/kg³ Vitamin K₁ equivalent

TPN Diets for the Determination of Arginine Adequacy,
Urea Cycle Activities and Arteriovenous Differences

Composition of TPN Diets:

<u>Compound (mL)</u>	<u>% Arginine</u>					
	<u>1.60</u>	<u>2.50</u>	<u>3.26</u>	<u>4.86</u>	<u>Devold</u>	<u>4.5</u>
Base AA Solution ^a	111	109	107	103	116	104
Arg Solution ^b	3.25	5.0	6.5	9.5	0	8.5
20% Lipid ^c	40.3	40.3	40.3	40.3	40.3	40.3
70% Dextrose ^d	91.4	91.4	91.4	91.4	91.4	91.4
Macroelements ^e	22.2	22.2	22.2	22.2	22.2	22.2
Vitamins & Trace Elements ^f	2.1	2.1	2.1	2.1	2.1	2.1
Water ^g	4.75	5.0	5.5	6.5	3.0	6.5

<u>Nutrient Density:</u>	(per 275 mL TPN Solution)
Non-N Kcal	299.05
g N	1.45
Non-N Kcal/g N	206.2

^aBase Amino Acid Solution; as outlined below

^bArginine Solution; 6.12 g arg-HCl/100 mL

^cIntralipid 20% (Kabivitrum, Inc., Alameda, CA); composition listed below

^d70% Dextrose; 70% Dextrose Injection, USP (Travenol Laboratories, Inc., Deerfield, IL). 70 g Dextrose, Hydrous, U.S.P., pH adjusted with hydrochloric acid, approx. pH 4.0. Approx. mOsmol/L = 3530.

^eMacroelements; as listed below

^fVitamins and trace elements; composition listed below

^gSterile water for injection, USP (Travenol Laboratories, Inc., Deerfield, IL)

^aBase Amino Acid Solution

Mixing Order	AA	Mw	% N/100 mL	g AA/100 mL
1	Met	149.21	0.0384	0.409
2	Ile	131.18	0.0418	0.391
3	Ser	105.10	0.1068	0.8016
4	Gly	75.05	0.3025	1.6219
5	Leu	131.18	0.0567	0.531
6	Trp	204.23	0.0149	0.109
7	Phe	165.19	0.0436	0.514
8	Val	117.15	0.0514	0.430
9	Thr	119.12	0.0422	0.359
10	Lys-HCl ₂	205.24	0.1228	0.641
11	Ala	89.10	0.1699	1.0813
12	Glu	147.13	0.1028	1.080
13	Pro	115.13	0.0538	0.442
14	Asp	133.11	0.0464	0.441
15	His	155.16	0.0571	0.211
16	Cys-HCl-H ₂ O	175.64	0.0015	0.013
17	Tyr	181.19	<u>0.0043</u>	<u>0.056</u>
			1.2570	9.1318

^c20% Lipid

Intralipid 20% (KabiVitrum, Inc., Alameda, CA)

Each 100 mL contains:

Soybean Oil	20.0 g
Phospholipids (from powdered egg yolk)	1.2 g
Glycerin, USP	2.25 g
Water for injection	q.s.
Calories	200

^eMacroelements:

Element	meq provided/ L TPN Solution	mL added/ 275 mL TPN Solution
Ca ²⁺ (1)	16	9.4
PO ₄ ⁼ (2)	16	0.6
Mg ²⁺ (3)	4	0.3
NaCl ⁽⁴⁾	40	2.8
K ⁺ (5)	80	9.7

(1)Ca²⁺ Calcium Gluconate Injection, USP
 (LyphoMed, Rosemont, IL)
 10% calcium gluconate, 0.465 mEq Ca²⁺/L

- (2)^{PO₄⁼} Potassium Phosphate
(Abbott Laboratories, North Chicago, IL)
45 mM PO₄⁼ and 66 mEq K⁺
- (3)^{Mg²⁺} Magnesium Sulfate Injection, USP
(SoloPak, Laboratories, Franklin Park, IL)
40.6 mEq Mg. pH adjusted with NaOH or H₂SO₄.
- (4)^{NaCl} Sodium Chloride Injection, USP 23.4%
(LyphoMed, Melrose Park, IL)
4 mEq/mL
- (5)^{K⁺} Potassium Acetate Injection, USP
(Abbott Laboratories, North Chicago, IL)
40 mEq K⁺

^fVitamins:

LyphoMed M.V.C. 9+3 (LyphoMed, Melrose Park, IL)

<u>Vitamin</u>	<u>provision/ 10 mL vial</u>
Ascorbic Acid (C)	100 mg
Vitamin A retinol ^a	3300 IU
Vitamin D (ergocalciferol) ^b	200 IU
Thiamine-HCl	3.0 mg
Riboflavin 5'-phosphate	3.6 mg
Pyridoxin-HCl	4.0 mg
Nicotinamide	40.0 mg
Pantothenic acid	15.0 mg
Vitamin E ^c	10 IU
Biotin	60 µg
Folic Acid	400 µg
Vitamin B ₁₂ (cyanocobalamin)	5 µg

^a991 retinol equivalents

^b5 µg ergocalciferol

^c6.7 d-alpha tocopherol equivalents

30% propylene glycol and 1% gentisic acid ethanolamide
2.4% polysorbate-20, 0.0009% butylated hydroxytoluene and
0.0002% butylated hydroxyanisol
pH adjusted with sodium hydroxide, sodium citrate and/or
citric acid
water for injection q.s.

^fTrace Elements

LyphoMed M.T.E.-5 (LyphoMed, Melrose Park, IL)

<u>Element</u>	<u>provision/mL</u>
Zn	5.0 mg
Cu	1.0 mg
Mn	0.5 mg
Cr	10.0 µg
Se	60.0 µg

0.9% Benzyl alcohol

pH approx. 2.5 adjusted with nitric acid or sodium hydroxide

Water for injection q.s.

sterile, non pyrogenic

Dietary compositions for experiments conducted for Objective 1; 'Development of a TPN Model for Determining Arginine Adequacy'.

Unsupplemented Lactalbumin Diet:

<u>Compound</u>	<u>g/Kg Diet</u>
Lactalbumin	165.7
Corn Oil	100.0
AIN 76A Mineral Mix	50.0
AIN 76A Vitamin Mix	5.0
Choline-Chloride	2.0
Non-Nutritive Cellulose Fiber	10.0
Sucrose	222.4
Corn Starch	444.9

Nutrient Density:

per Kg Diet

Non-N Energy	3569.1
g N	17.8
Non-N Kcal/g N	200.51

Preliminary TPN Diet:

<u>Compound (mL)</u>	<u>+Arg</u>	<u>-Arg</u>
Base AA Solution ^a	85.0	85.0
Arginine Solution ^b	10.0	---
Isonitrogenous Solution ^c	---	10.0
20% Lipid ^d	44.0	44.0
70% Dextrose ^e	110.5	110.5
Macroelements ^f	26.1	26.1
Vitamins & trace elements ^g	2.1	2.1
Water ^h	22.3	22.3

Nutrient Density:

(per 300 mL TPN solution)

Non-N Kcal	372.4
g N	1.11
Non-N Kcal/g N	335.5

^aBase Amino Acid Solution (8% TAA); as outlined below

^bArginine Solution; 3.22 g Arg/100 mL

^cIsonitrogenous Solution; 2.01 g Ala + 1.78 g Ser
+ 2.56 g Gly/100 mL

^{d,e,f,g,h} Remaining solutions were from the same sources as listed previously. Macroelements, vitamins and trace elements were added at same rate as listed previously.

^aBase Amino Acid Solution (8% TAA)

<u>Mixing Order</u>	<u>AA</u>	<u>% N/100 mL</u>	<u>g AA/100 mL</u>
1	Met	0.0307	0.327
2	Ile	0.0334	0.313
3	Ser	0.0960	0.720
4	Gly	0.2717	1.456
5	Leu	0.0453	0.425
6	Trp	0.0119	0.087
7	Phe	0.0349	0.411
8	Val	0.0411	0.344
9	Thr	0.0338	0.287
10	Lys-acetate	0.0983	0.513
11	Ala	0.1526	0.971
12	Glu	0.0823	0.864
13	Pro	0.0431	0.354
14	Asp	0.0371	0.353
15	His	0.0458	0.169
16	Cys-HCl-H ₂ O	0.0012	0.0103
17	Tyr	<u>0.0035</u>	<u>0.045</u>
		1.1660	7.6493

TPN composition for the orally-fed TPN diet experiment.

Orally-Fed TPN Diets:

<u>Compound (mL)</u>	<u>0.78% Arg</u>	<u>3.23% Arg</u>
AA Base Solution ^a	97.5	97.5
0.78% Arg Solution ^b	17.5	---
3.23% Arg Solution ^c	---	35.0
IsoN solution ^d	17.5	17.5
70% Dextrose ^e	128.0	128.0
20% Lipid Emulsion ^f	56.5	56.5
Macroelements ^g	36.5	36.5
Trace Elements & Vitamins ^h	3.0	3.0

<u>Compound (mL)</u>	<u>1.61% Gln</u>	<u>5.36% Gln</u>
AA Base Solution ^a	97.5	97.5
1.61% Gln Solution ⁱ	17.5	---
5.36% Gln Solution ^j	---	35.0
IsoN Solution ^d	17.5	---
70% Dextrose ^e	128.0	128.0
20% Lipid Emulsion ^f	56.5	56.5
Macroelements ^g	36.5	36.5
Trace Elements & Vitamins ^h	3.0	3.0

<u>Nutrient Density</u>	<u>per 55 mL Diet</u>
Non-N Energy	58.02
g N	0.290
Non-N Kcal/g N	200.09

^a Amino Acid Base Solution; as outlined below

^b 0.78% Arginine Solution; 0.460 g Arg-HCl/100 mL

^c 3.23% Arginine Solution; 0.860 g Arg-HCl/100 mL

^d IsoNitrogenous Solution; 0.544 g Ala + 0.475 g Ser
+ 0.686 g Gly/100 mL

ⁱ 1.61% Glutamine Solution; 0.72 g Gln/100 mL

^j 5.36% Glutamine Solution; 1.445 g Gln/100 mL

e,f,g,h Solutions were from the same sources as listed previously. Macroelements, vitamins and trace elements were added at same rate as listed previously.

^a Amino Acid Base Solution

<u>Amino Acid</u>	<u>g/L solution</u>
Met	4.09
Ile	3.91
Ser	8.016
Gly	16.219
Leu	5.31
Trp	1.09
Phe	5.14
Val	4.30
Thr	3.59
Lys-acetate	6.41
Ala	10.813
Glu	10.80
Pro	4.42
Asp	4.41
His	2.11
Cys-HCl-H ₂)	0.13
Tyr	<u>0.56</u>
	91.32

TPN compositions for experiments three and four in Objective 1.

TPN Diets:

<u>Compound (mL)</u>	<u>0.78% Arg</u>	<u>3.23% Arg</u>	<u>1.61% Gln</u>	<u>5.36% Gln</u>	<u>-Arg</u>
Base AA Solution ^a	72.30	69.55	72.30	69.55	75.05
Arg Solution ^b	12.50	25.00	---	---	---
Gln Solution ^c	---	---	12.50	25.00	---
20% Lipid ^d	40.30	40.30	40.30	40.30	40.30
70% Dextrose ^e	91.40	91.40	91.40	91.40	91.40
Macroelements ^f	22.70	22.70	22.70	22.70	22.70
Vitamins & Trace Elements ^g	2.10	2.10	2.10	2.10	2.10
Water ^h	33.70	23.95	33.70	23.95	45.45

^a Base Amino Acid Solution; same formula as that for orally-fed TPN diets.

^b Arginine Solution; 0.86 g Arg-HCl/100 mL H₂O

^c Glutamine Solution; 1.44 g Glu/100 mL H₂O

^{d,e,f,g,h} Remaining solutions from the same sources as listed previously. Vitamins, trace elements and macroelements were added at the same rate as listed previously.

APPENDIX II METHODS

This appendix contains the procedural outline for the analyses performed during this research.

AutoAnalyzer and Methods

The AutoAnalyzer consists of six basic modules which are the sampler, proportioning pump with manifold, dialyzer, heating bath, colorimeter and recorder. The sampler holds up to 40 samples and is cam operated to aspirate samples or wash water at pre-set time intervals. Pre-set cams determine the number of samples aspirated per hour as well as the ratio of sampling and wash time. A one second aspiration of air between sample and wash provides air segments which form an anti-diffusion barrier between samples and wash fluids. The proportioning pump with manifold introduces, proportions, mixes samples and reagents and moves these fluids to the other modules of the system. The pump consists of a chain-roller assembly which is driven by a constant-speed gear motor and a platen. The manifold consists of up to 14 plastic tubings of specific internal diameters, fittings and mixing coils. The tubes are placed between the chain-roller assembly and the platen which allows the chain-roller assembly to simultaneously and uniformly occlude the

individual tubes and provide a fixed delivery of sample, reagents and gases to the system. The membrane dialyzer may or may not be used depending on the analysis to be performed. The dialyzer consists of a 37°C constantly stirred water bath and a membrane (40-60 angstroms) sandwiched between dialyzer plates. Generally, the dialyzer is used to separate ions or smaller organic molecules from larger molecules and proteins. A variable (25°F-95°F) or fixed temperature oil heating bath generally equipped with a 40 ft coil allows time for chromophore development. A filter colorimeter equipped with a single light source in which twin beams are provided measures the intensity of the developed chromophore. One beam passes through the sample contained in a 15 mm tubular flowcell to a permanently stable photocell and the second passes to an identical reference photocell. The ratio of the sample to the reference voltage is continuously measured in a null balance system and recorded on a moving chart by the recorder.

Automated Determination of Orotic AcidReagents0.2 M Citrate Buffer, pH 2.5

Potassium Citrate, monohydrate	64.8 g
Citric acid, monohydrate	42.0 g
d-d-H ₂ O, q.s.	1000 mL

Dissolve potassium citrate, then citric acid in approximately 700 mL water. Adjust the pH to 2.5 with approximately 35 mL concentrated HCl, then q.s. to 1 liter.

Reducing Reagent

L-ascorbic acid	50.0 g
d-d-H ₂ O, q.s.	1000 mL

Dissolve ascorbic acid in approximately 900 mL water, then q.s. to 1 L. Store in a brown bottle.

Color Developer (Erllich's Reagent)

p-dimethylaminobenzaldehyde	4.7 g
n-propanol, q.s.	250 mL

Dissolve p-dimethylaminobenzaldehyde in approximately 200 mL n-propanol then, q.s. to 250 mL. Filter through a Whatman #1 filter and store in a brown bottle.

Saturated Bromine Water

d-d-H ₂ O	250 mL
Bromine	1 mL

Prepare solution under a fume hood. Add water to a brown bottle, slowly add bromine down the side of the bottle stirring constantly.

Orotic Acid Standards

Desiccated Orotic Acid	6.4 mg
d-d-H ₂ O	200 mL

Dissolve orotic acid in approximately 180 mL water, then q.s. to 200 mL making 3.2 mg% orotic acid. Take 100 mL and dilute to 200 mL making 1.8 mg%. Continue diluting the standard in the same manner until the concentration is 0.1 mg%.

Figure 35 displays the manifold configuration for the determination of orotic acid.

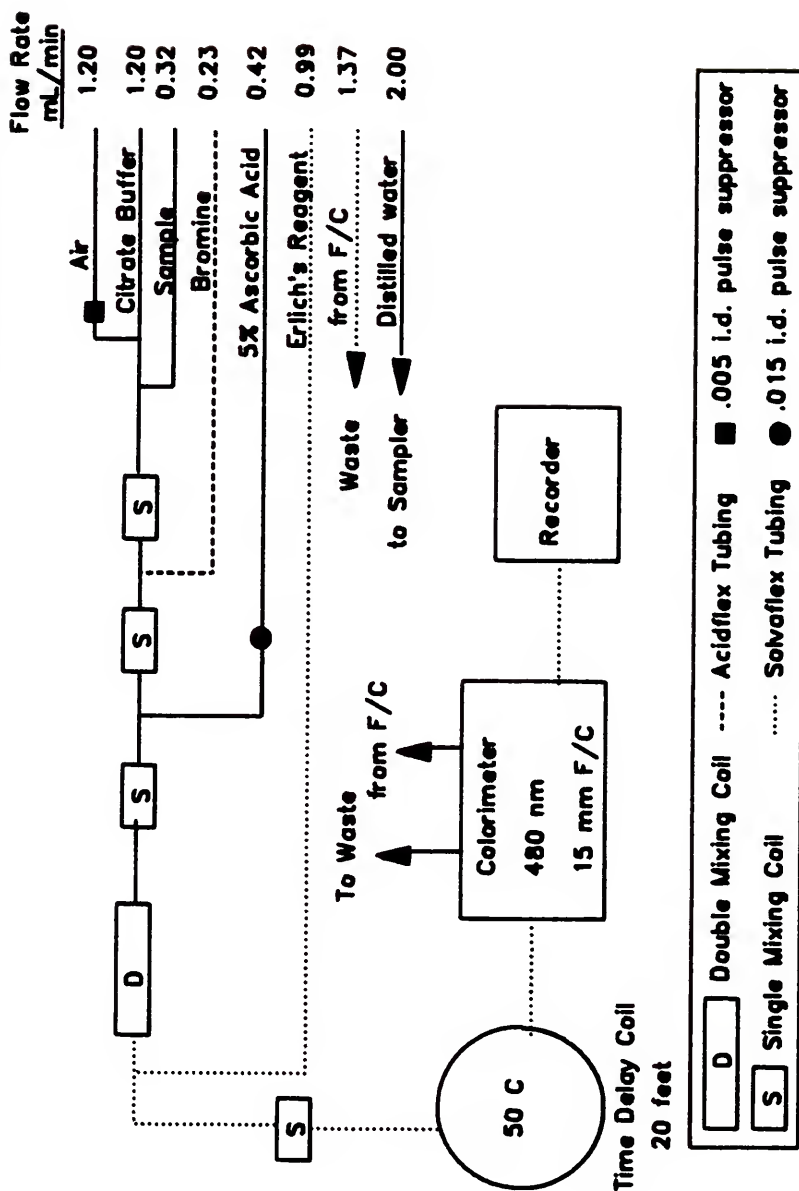


Figure 35. Manifold for the Determination of Urinary Orotic Acid.

Automated Determination of Urinary Urea NitrogenReagents0.9% Sodium Chloride

Sodium Chloride	9.0 g
d-d-H ₂ O, q.s.	1000 mL
Brij-35	1.0 mL

Dissolve sodium chloride in approximately 750 mL water, q.s. to 1000 mL. Add Brij-35 and store refrigerated.

Urinary Urea Nitrogen 'Color'

2,3-Butanedione monoxime (DAMO)	3.35 g
Thiosemicarbazide	0.67 g
d-d-H ₂ O, q.s.	2000 mL
30% Brij-35	1.0 mL

Dissolve DAMO in approximately 1500 mL, add and dissolve thiosemicarbazide, then q.s. to 2000 mL with d-d-H₂O. Add 1.0 mL Brij-35 and store in brown bottle. Immediately before use, filter UUN 'color' through a Whatman #1 filter.

Urinary Urea Nitrogen 'Acid'

Ferric chloride	30.0 g
Phosphoric acid, 85%,	600 mL
20% sulfuric acid, q.s.	2000 mL

Dissolve ferric chloride in a beaker with approximately 200 mL d-d-H₂O. Measure phosphoric acid in a graduated cylinder, add ferric chloride solution and q.s. to 900 mL with d-d-H₂O. Transfer solution to 2 L volumetric flask and q.s. with 20% sulfuric acid. Store in brown bottle.

1000 mg% Stock Standard

Dessicated urea granules	4.282 g
0.01 N H ₂ SO ₄ , q.s.	200 mL

Dissolve urea granules in approximately 150 mL 0.01 N H₂SO₄, then q.s. to 200 mL.

Working Standards

	mL Stock Standard
100 mg%	10.0
200 mg%	20.0
400 mg%	40.0
600 mg%	60.0

Add indicated mL of stock standard to 100 mL volumetric flask and q.s. with 0.01 N H_2SO_4 .

Figure 36 shows the manifold configuration for the determination of urinary urea nitrogen.

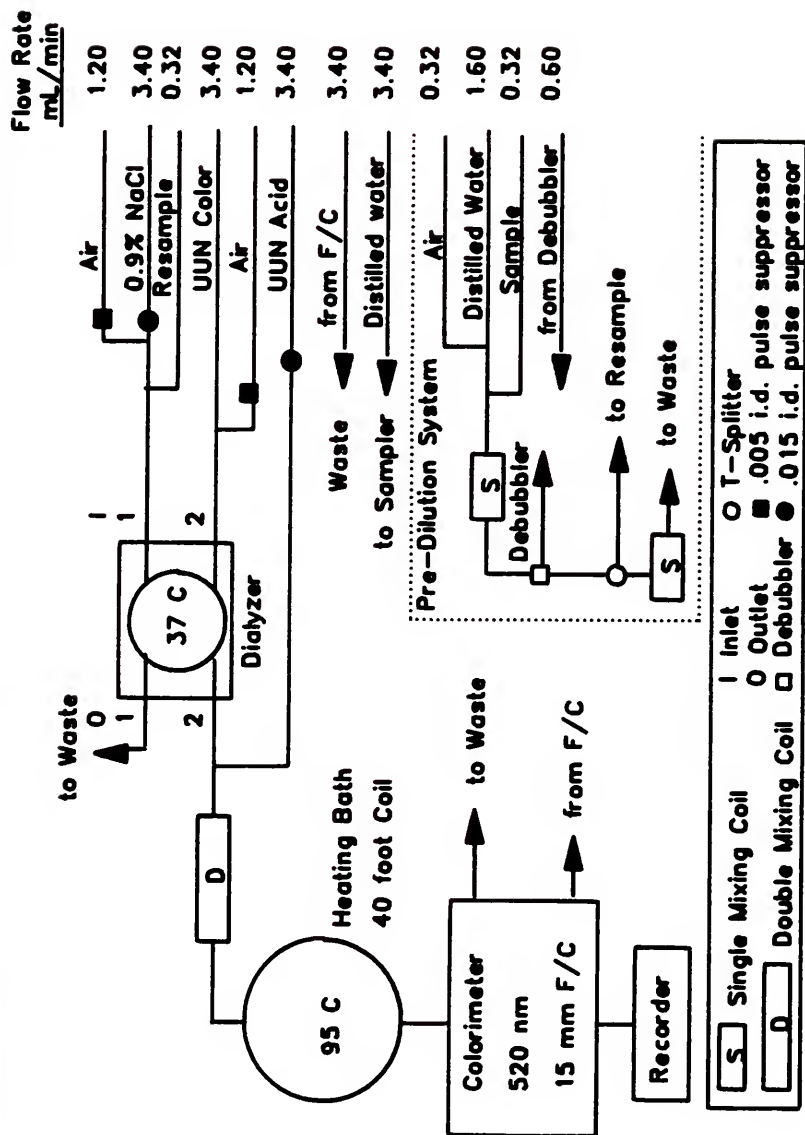


Figure 36. Manifold for the Determination of Urinary Urea Nitrogen.

Semi-Automated Determination of Kjeldahl NitrogenDigestion Mixture

Selenium Dioxide	3.0 g
Perchloric acid, 70%	7.5 mL
Sulfuric acid, 98%, q.s.	1000 mL

Prepare under a fume hood. The solution is extremely corrosive, thus one should wear safety glasses. Dissolve selenium dioxide in approximately 50 mL d-d-H₂O, slowly add perchloric acid and then, slowly add sulfuric acid to 1 L. Allow to cool overnight, adjust the volume, if necessary, with sulfuric acid. Store in brown glass bottle or Repipette.

Reagents35% Sodium Hydroxide

Sodium Hydroxide	350 g
Sodium-Potassium Tartrate	50 g
d-d-H ₂ O, q.s.	1000 mL

Dissolve sodium hydroxide in approximately 700 mL water, add and dissolve sodium-potassium tartrate and then q.s. to 1 L. Store in plastic.

Alkaline Phenol

phenol crystals	12.5 g
sodium nitroferricyanide	0.1 g
20% NaOH, q.s.	100 mL

Make fresh daily and wear gloves while handling phenol crystals. Dissolve phenol crystals in approximately 90 mL 20% sodium hydroxide, add and dissolve nitroferricyanide, then q.s. to 100 mL. Filter through Whatman #1 filter paper into an Erlenmeyer flask. Wrap the flask with foil.

Sodium Hypochlorite

Use commercially available bleach having 5% available chlorine. Store in refrigerator.

Stock Standard

Dessicated Ammonium Sulfate	0.09434 g
2% sulfuric acid, q.s.	200 mL

Working Standards

	mL Stock Standard
0.25 mg% N	2.5 mL
0.5 mg% N	5.0 mL
1.0 mg% N	10.0 mL
2.0 mg% N	20.0 mL
3.0 mg% N	30.0 mL
4.0 mg% N	40.0 mL

Add the indicated volume of stock standard and q.s. to 100 mL with 2% sulfuric acid.

Digestion of Samples

Samples are placed in 100 mL Kjeldahl flasks with the appropriate amount of digestion mixture. Up to 2 mL of 30% peroxide is added to samples containing significant quantities of fat or carbohydrate to speed the oxidation of carbon compounds to CO₂. Kjeldahl flasks are placed on a specially designed burner (Labconco, Inc., Kansas City, MO) and heated for a specified time. Most samples darken upon heating and the digestion must continue for a specified time once the solution has become colorless which indicates oxidation of all carbon compounds to CO₂. Weights of samples and digestion time after oxidation of carbon compounds is indicated for each constituent measured. Once digestion is complete, the flasks are removed and allowed to cool. Samples are diluted to 2% sulfuric acid and an aliquot is assayed on the AutoAnalyzer for total nitrogen.

Urine. One-half mL urine plus 2 mL digestion mixture are digested for 16 minutes.

Organ Tissue. Accurately weighed ground sample between 0.05 and 0.10 g plus 10 mL digestion mixture are digested for 40 minutes.

Semipurified Diet. Accurately weighed sample between 0.05 and 0.10 g plus 10 mL digestion mixture are digested for 40 minutes.

Lactalbumin Protein. Accurately weighed sample between 0.025 and 0.05 g plus 10 mL digestion mixture are digested for 40 minutes.

TPN Diets. One-half mL diet plus 2 mL digestion mixture are digested for 40 minutes.

Controls. For urine samples, 2.5 mL of 100 mg% urea plus 2 mL digestion mixture are digested for 16 minutes. For solid samples, 98-99% pure BSA is accurately weighed between 0.005 and 0.01 g plus 10 mL digestion mixture are digested for 40 minutes.

Figure 37 shows the manifold configuration for the determination of Kjeldahl nitrogen.

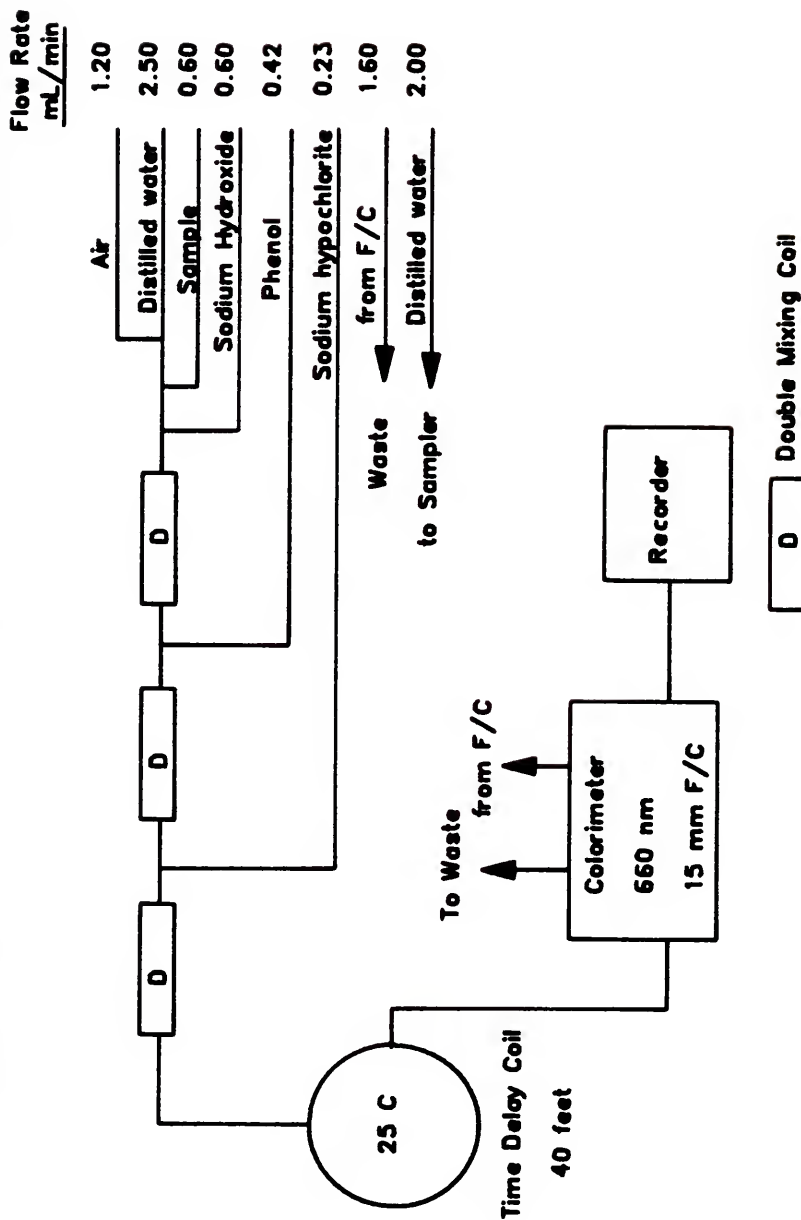


Figure 37. Manifold for the Semi-Automated Determination of Kjeldahl Nitrogen.

Automated Determination of Urinary CreatinineReagents0.9% Sodium Chloride

Sodium Chloride	9.0 g
d-d-H ₂ O, q.s.	1000 mL
Brij-35	1.0 mL

Dissolve sodium chloride in approximately 750 mL water, q.s. to 1000 mL then add Brij-35. Store refrigerated.

2.0% Sodium Hydroxide

Sodium Hydroxide	20.0 g
d-d-H ₂ O, q.s.	1000 mL

Dissolve sodium hydroxide in approximately 750 mL water, q.s. to 1000 mL.

Saturated Picric Acid

Picric Acid	13.0 g
d-d-H ₂ O, q.s.	1000 mL

Add picric acid to 1 L volumetric flask, q.s. to 1000 mL and stir at least one hour. Let solution stand overnight, filter through a Whatman #1 filter paper into a one liter polyethylene storage container.

Standards

100 mg% Stock standard

dessicated creatinine crystals	100	mg
0.1 N HCl, q.s.	100	mL

Working Standards

mL Stock Standard

5 mg%	5
10 mg%	10
20 mg%	20

q.s. all working standards to 100 mL with 0.1 N HCl

See Figure 38 for the manifold configuration for the determination of urinary creatinine.

Flow Rate
mL/min

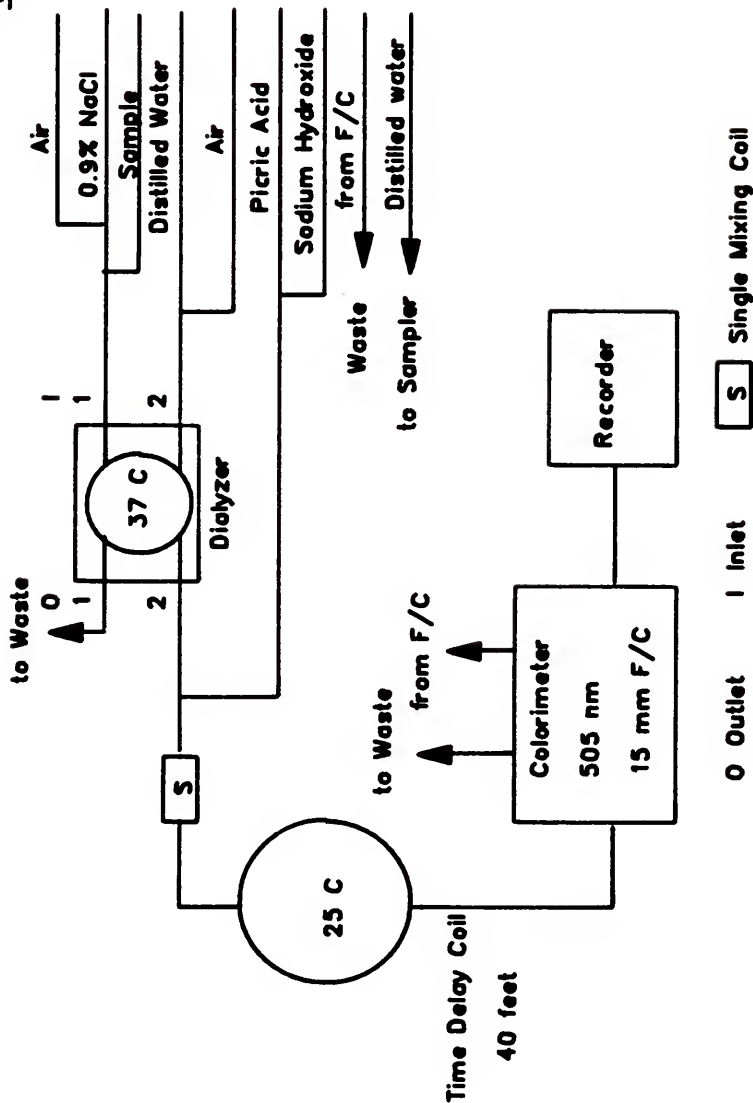


Figure 38. Manifold for the Determination of Urinary Creatinine.

Citric Acid DeterminationSpectrophotometric Method of Camp and Farmer (1967)ReagentsPotassium permanganate-sodium bromide solution

potassium permanganate	5.0 g
sodium bromide	5.0 g
d-d-H ₂ O, q.s.	100 mL

Dissolve potassium permanganate in approximately 85 mL boiled, cooled water, then add and dissolve sodium bromide and q.s. to 100 mL with boiled, cooled water. Store in brown bottle.

Sulfuric acid-metaphosphoric acid

metaphosphoric acid (HPO ₃)	5.0 g
9 N sulfuric acid	50.0 mL

Dissolve metaphosphoric acid in sulfuric acid. Store in glass bottle.

Hydrogen peroxide

Use commercially available 3% or dilute concentrated 30% peroxide to 3%.

Thiourea solution, pH 9.2

thiourea	4.0 g
d-d-H ₂ O, q.s.	100 mL
sodium borate	2.0 g

Dissolve thiourea in approximately 85 mL water, then q.s. to 100 mL. Add sodium borate to the 4% thiourea solution.

n-Heptane

Use commercially available n-heptane.

Procedure

Place 1.0 mL of urine or standard in a 13 mL Teflon-lined capped glass test tube, add 1.0 mL of the sulfuric acid-metaphosphoric acid, cap and place in ice bath. While gently shaking, add 2.0 mL of the potassium permanganate-sodium bromide solution. Return the tube to the ice bath. After 10 minutes, add 3% peroxide in a dropwise manner until the solution becomes colorless. (Note: bromine gas is evolved during this process, thus this step must be done under a fume hood) Add 1.5 mL n-heptane to the tube and vortex for 30 s.

Transfer a 1.0 mL aliquot of the n-heptane layer to a second Teflon-lined capped glass test tube and add 3.5 mL of the thiourea solution. Vortex at the highest speed for 1 minute. Transfer the aqueous phase to a cuvette and read at 445 nm. The blank consists of 1.0 mL n-heptane and 3.5 mL thiourea which is vortexed and the aqueous portion is removed.

A standard curve is developed and unknown concentrations are read off the curve.

Enzymatic Method of Nielsen (1976)

Reagents

1 M TRIZMA-HCl, pH 8.2

Tris-(hydroxymethyl) aminomethane	60.57 g
d-d-H ₂ O, q.s.	500 mL

Dissolve TRIZMA-HCl in approximately 250 mL H₂O and adjust the pH to 8.20 with 2 M HCl. Transfer the solution to a 500 mL volumetric flask and add d-d-H₂O to 500 mL. Store in refrigerator.

0.15 M TRIZMA-HCL, Ph 8.2

1 M TRIZMA-HCl, pH 8.2	150 mL
d-d-H ₂ O, q.s.	1000 mL

Add 150 mL 1 M TRIZMA-HCl to a 1 L volumetric flask and bring to volume with d-d-H₂O. Store in refrigerator.

0.4 mM Zinc Sulfate

Zinc sulfate ($\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$)	143.78 mg
d-d- H_2O , q.s.	500 mL

Dissolve ZnSO_4 in approximately 400 mL water, then q.s. to 500 mL.

0.50 mM NADH

β -Nicotinamide adenine dinucleotide	17.74 mg
d-d- H_2O , q.s.	50 mL

Make fresh daily in a volume appropriate for the number of assays; this amount provides 100 assays. Dissolve NADH in approximately 40 mL water, then q.s. to 50 mL.

Malate Dehydrogenase Suspension (MDH)

Malate Dehydrogenase (MDH)	1 mg
d-d- H_2O , q.s.	10 mL

Make fresh daily. Dissolve 1 mg MDH in enough water, then add to 10 mL.

Citrate Lyase Suspension (CL)

Citrate Lyase (CL)	30 mg
0.15 M TRIZMA-HCl, pH 8.2	2.0 mL

Make fresh daily in a volume appropriate for the number of assays; this amount provides 100 assays. The amount in one sample should be equivalent to 0.3 mg CL powder/0.02 mL 0.15 M TRIZMA-HCl. Dissolve appropriate amount of CL in appropriate volume of 0.15 M TRIZMA-HCl. TRIZMA should be at 4 C and the suspension should be kept at 4 C. Suspension is stable for up to 6 hours at this temperature.

'Mixed Cuvette Reagent'

1.0 M TRIZMA-HCl, pH 8.2	5.0 mL
0.4 mM ZnSO_4	50.0 mL
0.5 mM NADH	40.0 mL
MDH	5.0 mL

Make 'mixed cuvette reagent' daily in a volume appropriate for the number of assays; this amount provides 100 assays. Mix the solutions in an appropriately sized beaker and keep solution on ice until use.

Citric Acid StandardsProcedureCuvette Mixture

0.15 M TRIZMA-HCl, pH 8.2	0.9 mL
'Mixed Cuvette Reagent'	1.0 mL
urine or standard	0.1 mL

Add the above volumes to each cuvette and allow to come to room temperature (about 10 min). Read each cuvette against an air blank at 340 nm. Add 0.02 mL CL to each cuvette. Cover with plastic cap and invert to mix (an alternative is to mix with a plastic rod). Allow the reaction to come to completion which is accomplished in 5 to 8 minutes. Read each cuvette again at 340 nm to determine the reduction in absorbance.

Determine the difference in absorbance for standards and plot a standard curve. Determine the difference in absorbance for urines and then determine the concentration of citric acid in the samples from the standard curve.

Urea Cycle Enzyme Activity DeterminationsCarbamoyl Phosphate Synthetase (CPS)Reagents1.0 M Ammonium Bicarbonate

NH_4HCO_3	7.91 g
d-d- H_2O , q.s.	100 mL

Dissolve NH_4HCO_3 in approximately 90 mL water, then q.s. to 100 mL.

0.1 M N-Acetyl-L-Glutamate, pH 7.2

N-Acetyl-L-Glutamate (NAG)	1.89 g
d-d- H_2O , q.s.	10 mL

Dissolve NAG in approximately 9 mL water and adjust the pH to 7.2 with . Transfer solution to a 10 mL volumetric flask and add water.

0.1 M L-Ornithine

L-ornithine	1.32 g
d-d- H_2O , q.s.	100 mL

Dissolve ornithine in approximately 90 mL water, then q.s. to 100 mL.

0.1 M ATP, pH 7.2

ATP, disodium salt	5.51 g
d-d- H_2O , q.s.	100 mL

Dissolve ATP in approximately 90 mL water, then q.s. to 100 mL.

0.2 M Magnesium Sulfate

MgSO_4	2.41 g
d-d- H_2O , q.s.	100 mL

Dissolve MgSO_4 in approximately 90 mL water, then q.s. to 100 mL.

0.1 M Phosphoenolpyruvate (PEP)

PEP, trisodium salt	2.34 g
d-d-H ₂ O, q.s.	100 mL

Dissolve PEP in approximately 90 mL water, then q.s. to 100 mL.

Ornithine Carbamoyl Transferase

15,000 U/mL (U is defined as μmol product/hour)

The isolation procedure for OCT is outlined at the end of the section for urea cycle enzyme activities determination.

Pyruvate Kinase

200,000 U/mL

A commercial source for pyruvate kinase was used.

Reagent Pool (provides 6 working pools)

<u>Compound</u>	<u>mL/pool</u>
1.0 M NH ₄ CO ₃	1.5
0.1 M NAG, pH 7.2	1.5
0.1 M L-ornithine	1.5
0.1 M ATP, pH 7.2	3.6
0.2 M MgSO ₄	3.6
0.1 M PEP	6.0
d-d-H ₂ O	1.8

Mix all solutions together, divide into 3.0 mL aliquots and store frozen in appropriately sized plastic vials. Each aliquot is designated as a 'working pool'.

Assay Pool (sufficient for 15 assays)

<u>Component</u>	<u>mL/pool</u>
working pool	3.0
OCT	0.05
Pyruvate kinase	0.02

Thaw working pool on ice. Once the pool is thawed, bubble CO₂ through the solution for about 2 min to bring the pH to 7.0. Add supplementary enzymes and keep on ice.

Assay Procedure

Equipment

15 mL glass conical tubes	centrifuge
37°C water bath	ice bath

Prior to homogenization of tissue to be analyzed for CPS activity, place sufficient conical tubes in a rack for a blank and duplicate 'test' assays. A 'blank' is a 0-time assay in which the enzyme reaction is terminated immediately; whereas a 'test' assay allows the enzyme reaction to proceed for 15 min. Place 0.2 mL of the assay pool to each tube and keep the tubes on ice until use.

Procedure

1. Homogenize liver sample in 19 volumes ice cold water. No further dilution of the tissue is necessary.
2. Initiate the reaction by adding 0.1 mL of the tissue homogenate. Shake the tube to mix and immediately place the tube in a 37°C water bath.
3. The 'blank' is a 0-time incubation. Thus immediately after the tissue homogenate is added to the assay pool, terminate the reaction by adding 0.5 mL 8% TCA. Place the 'blank' in the 37°C water bath also.
4. The reaction is terminated after a 15 min incubation period by adding 0.5 mL 8% TCA.
5. Centrifuge samples at 1000 rpm for approximately 5 min or until the precipitated protein has pelleted to the bottom of the conical tube.

Determination of Citrulline Production

Reagents

Acid-Ferric Solution

d-d-H ₂ O	550 mL
Sulfuric Acid (98%)	250 mL
Phosphoric Acid (85%)	200 mL
Ferric Chloride (FeCl ₃)	250 mg

Add acids to water and cool to room temperature. Dissolve FeCl_3 into acid solution. Store in a brown bottle. The solution is stable for 2 months.

Diacetylmonoxime (DAMO)

Diacetylmonoxime (2,3-butanedione monoxime)	500 mg
d-d- H_2O , q.s.	100 mL

Dissolve DAMO in approximately 90 mL water, then q.s. to 100 mL. Store in a brown bottle. The solution is stable approximately 1 month.

Chromogenic Reagent

DAMO	50 mL
Ferric-Acid Solution	100 mL
Thiosemicarbazide	5 mg

Prepare immediately before use; solution is stable for approximately 1 hr. Dissolve thiosemicarbazide in DAMO, then add Acid-Ferric solution.

Citrulline Standards

30 mMol Citrulline Stock Standard

Citrulline	525.6 mg
d-d- H_2O , q.s.	100 mL

Dissolve citrulline in approximately 90 mL water, then q.s. to 100 mL.

Working Standards

<u>Concentration</u>	<u>μL Stock Standard</u>
300 μmol	10
900 μmol	30
1800 μmol	60
3000 μmol	100

Add volumes indicated for each working standard to a 100 mL volumetric flask and q.s. each to 100 mL.

Citrulline Determination Procedure

Equipment

Boiling water bath	Vortex
5 mL Pyrex test tubes	

Procedure

Supernatant from CPS assay, or Standard	0.1 mL
Chromogenic reagent	3.0 mL

Put supernatant from all assays into respective test tubes. As rapidly as possible, add Chromogenic reagent to each test tube, vortex to mix and return to test tube rack. Cover test tubes with vented stoppers or marbles and place entire rack into boiling water bath. Cover entire water bath with foil and heat for 5 min. After 5 min, remove test tube rack, cool to room temperature and determine absorbance at 530 nm against a reagent blank consisting of 0.1 mL d-d-H₂O and 3.0 mL Chromogenic reagent.

Make a standard curve and determine the citrulline concentration of both 'blank' and 'test' assays. Enzyme activity is determined by:

Activity = $(\Delta C \times 0.8 \times 4) / 0.1 \times \text{mg protein (or g tissue) / assay}$.

where:

ΔC = difference in citrulline concentration between the 'blank' and the 'test' assays.

0.8 = the final volume of the assay

4 = the conversion of 15 min to 1 hr.

0.1 = volume of the supernatant taken for color development.

Argininosuccinate SynthetaseReagents0.1 M ATP, pH 7.2

prepared as for CPS assay

0.2 M Magnesium sulfate

prepared as for CPS assay

0.1 M Phosphoenolpyruvate

prepared as for CPS assay

0.1 M L-aspartate, pH 7.5

L-aspartate	1.33 g
d-d-H ₂ O, q.s.	100 mL

Dissolve aspartate in approximately 90 mL water, then q.s. to 100 mL.

1.0 M Potassium phosphate Buffer, pH 7.5

K ₂ HPO ₄	13.93 g
KH ₂ PO ₄	2.72 g
d-d-H ₂ O, q.s.	100 mL

Pyruvate Kinase

200,000 U/mL

A commercial source of pyruvate kinase was used.

Argininosuccinate Lyase (AL)

6,000 U/mL

Argininosuccinate lyase was isolated from bovine liver according to the outline at the end of this section on Urea cycle enzyme activity determinations.

Arginase

18,000 U/mL

A commercial source of arginase was used.

Reagent Pool (Sufficient for 7 working pools)Common Pool

<u>Solution</u>	<u>mL/pool</u>
0.1 M ATP, pH 7.2	1.2
0.2 M MgSO ₄	0.9
0.1 M PEP	7.5
0.1 M L-Aspartate, pH 7.5	2.0
1.0 M K-phosphate buffer	1.5

Mix above solutions together and divide in half (6.55 mL each) for a 'Test' pool and a 'Blank' pool.

<u>Solution</u>	<u>mL in 'Test'</u>	<u>mL in 'Blank'</u>
Common Pool	6.55	6.55
0.1 M L-citrulline	1.0	---
d-d-H ₂ O	---	1.0

Mix common pool and solutions necessary for 'Test' and 'Blank' pools. Divide each pool into 1.0 mL aliquots for their respective working pools and store frozen in appropriately sized plastic vials.

Assay Pool (sufficient for 6 assays)

<u>Component</u>	<u>mL in 'Test'</u>	<u>mL in 'Blank'</u>
working pool	1.0	1.0
pyruvate kinase	0.01	0.01
argininosuccinate lyase	0.10	0.10
arginase	0.2	0.20

Thaw working pool on ice, then add supplementary enzymes and keep assay pools on ice until use.

Assay ProcedureEquipment

15 mL glass conical tubes	centrifuge
37°C water bath	ice bath

Prior to tissue homogenization, place sufficient conical tubes in a rack for 'blank' and 'test' samples for each tissue to be analyzed for AS activity. Place a 0.2 mL aliquot of 'blank' or 'test' assay pool in appropriate tubes and keep on ice until use.

Procedure

1. Homogenize tissue sample in 19 volumes of ice cold water. No further dilution of liver or kidney is necessary.
2. Initiate reaction by adding 0.1 mL of homogenized tissue to conical tube and immediately place in 37°C water bath and incubate for 30 min.
3. After 30 min, terminate the reaction by adding 0.1 mL 20% TCA.
4. Centrifuge samples at 1000 rpm for approximately 5 min or until the precipitated protein has pelleted to the bottom of the conical tube.

Determination of AS Urea Production

Reagents

'Acid Reagent'

Sulfuric Acid (98%)	90 mL
Phosphoric Acid (85%)	270 mL
0.1 M FeCl ₃ (2.7 g/100 mL)	1 mL
d-d-H ₂ O, q.s.	1000 mL

Add acids and FeCl₃ to approximately 600 mL water, cool to room temperature, then q.s. to 1 L.

3% α-isonitrosopropiophenone

α-isonitrosopropiophenone	3 g
95% ethanol	100 mL

Dissolve isonitrosopropiophenone in ethanol and store in a brown bottle.

Urea Standards4 mM Stock Urea

Dessicated urea crystals	48.04 mg
0.1 M H ₂ SO ₄ , q.s.	200 mL

Dissolve urea in approximately 190 mL of 0.1 M H₂SO₄, then q.s. to 200 mL.

10 mM Stock Citrulline

Dessicated citrulline	875.95 mg
d-d-H ₂ O, q.s.	500 mL

Dissolve citrulline in approximately 490 mL water, then q.s. to 200 mL.

50% Trichloroacetic Acid (TCA)

TCA, dilute 100% TCA with d-d-H₂O to make 50% TCA.

Working Standards'Compensated Urea Standards'

Final Concentration

Urea (Citrulline)

<u>μmol/0.2 mL Std Sol'n</u>	<u>mL Stock Urea</u>	<u>mL Stock Citrulline</u>
0 (1.00)	0	50
0.02 (0.98)	2.5	49
0.08 (0.92)	10	46
0.16 (0.81)	20	42
0.24 (0.76)	30	38
0.32 (0.68)	40	34
0.40 (0.60)	50	30

Add indicated volumes of Stock Urea and Stock Citrulline to 100 mL volumetric flasks. Add 10 mL 50% TCA to each flask, then q.s. each flask to 100 mL with d-d-H₂O.

'Uncompensated Urea Standards'

<u>Final Urea Concentration</u> <u>μmol/0.2 mL Standard Solution</u>	<u>mL Stock Urea</u>
0	0
0.02	2.5
0.08	10
0.16	20
0.24	30
0.32	40
0.40	50

Add indicated volumes of Stock Urea to 100 mL volumetric flasks. Add 10 mL 50% TCA to each flask, then q.s. each flask to 100 mL.

AS Urea Determination ProcedureEquipment

13 x 100 mm Pyrex test tubes	boiling water bath
vented stoppers or marbles	

Procedure

Supernatant from AS assay, or standard	0.2 mL
Acid Reagent	2.0 mL
α-isonitrosopropiophenone	0.1 mL

Put 'Acid Reagent' and then supernatant from all assays into respective test tubes. As rapidly as possible, add isonitrosopropiophenone and vortex to mix. Cover test tubes with vented stoppers or marbles and place in boiling water bath. Cover entire water bath with foil and heat for 10 min. After 10 min, remove test tube rack, cool and determine the absorbance at 540 nm against a 0 urea, 0 citrulline reagent blank.

Make a standard curve for 'compensated' standards and 'uncompensated' standards. Determine the concentration of urea in the 'test' samples according to the 'compensated' standards and the 'blank' samples according to the 'uncompensated' standards.

Activity = $(\Delta U \times 0.4 \times 2) / (0.2 \times \text{mg protein})$ [or g
tissue]/assay

Where:

ΔU = difference in urea concentration between the
'test' and the 'blank'

0.4 = final assay volume (mL)

2 = correction to convert 30 min to 1 hr

0.2 = sample volume (mL)

Arginase (A)Reagents30 mM MnCl₂

MnCl ₂	593.7 mg
d-d-H ₂ O, q.s.	100 mL

Dissolve MnCl₂ in approximately 90 mL water, then q.s. to 100 mL.

300 mM L-glycine, pH 9.8

L-glycine	2.25 g
d-d-H ₂ O, q.s.	100 mL

Dissolve glycine in approximately 90 mL water, adjust the pH with approximately 2 mL of 20% NaOH and q.s. to 100 mL.

0.5% BSA

bovine serum albumin (BSA)	0.5 g
d-d-H ₂ O, q.s.	100 mL

Dissolve BSA in approximately 90 mL water being careful not to create too many bubbles, then q.s. to 100 mL.

375 mM L-arginine, pH 9.8

L-arginine	6.53 g
d-d-H ₂ O, q.s.	100 mL

Dissolve arginine in approximately 90 mL water, adjust the pH with approximately 2 mL 6 N HCl and q.s. to 100 mL with water.

20% Trichloroacetic Acid

dilute 20 mL of 100% TCA to 100 mL.

Activation Medium Mix immediately before use.

30 mM MnCl ₂	25 mL
300 mM L-glycine, pH 9.8	25 mL

Assay Procedure

Equipment

55°C water bath
13 x 100 mm test tubes
centrifuge

37°C water bath
15 mL conical glass tubes

Prior to tissue homogenization, prepare 13 x 100 mm test tube for each tissue for the preincubation step and put 2.0 mL of the activation medium in each tube. Also prepare conical tubes sufficient for a blank and test assays for each tissue. Put 0.2 mL of 375 mM L-arginine in each tube. It is not necessary to keep these tubes on ice as it was for CPS and AS assays.

Procedure

1. Homogenize liver and kidney in 19 volumes of ice cold water. Dilute the liver homogenate 1:25 and the kidney homogenate 1:5 with 0.5% BSA.
2. Preincubate each tissue by adding 0.5 mL of the tissue suspension to each preincubation tube. Incubate at 55 °C for 20 min.
3. Assay is initiated by adding 0.1 mL of the activated suspension to appropriate blank and test 15 mL conical tubes. Place tubes in 37°C water bath and incubate for 10 min. Blanks are made by adding 0.1 mL of 20% TCA immediately after the 0.1 mL of activated suspension. Blanks are incubated at 37°C also.
4. The reaction is terminated by adding 0.1 mL of 20% TCA.
5. Centrifuge samples at 1000 rpm for approximately 5 min or until the precipitated protein has pelleted in the bottom of the conical tube.

Determination of A Urea Production

Reagents

The same reagents as those used for AS urea determination are also used for A urea determination but with the following alterations.

Working Standard

<u>Final concentration</u> <u>μmol/0.2 mL Standard Sol'n</u>	<u>mL 4 mM urea</u>
0	0
0.02	2.5
0.08	10
0.16	20
0.24	30
0.32	40
0.40	50

To each 100 mL volumetric flask, add the indicated volume of 4 mM urea. In addition, to each flask, add 20 mL 375 mM arginine, 20 mL of 30 mM MnCl_2 and 10 mL of 50% TCA. Bring the volume to 100 mL for each flask.

A Urea Determination ProcedureEquipment

Boiling water bath	13 x 100 mm Pyrex test tubes
Vortex	vented stoppers or marbles

Procedure

Supernatant from A assay, or standard	0.2 mL
Acid Reagent	2.0 mL
isonitrosopropiophenone	0.1 mL

Add acid reagent and then appropriate supernatant to each test tube. As rapidly as possible, add the isonitrosopropiophenone and vortex to mix. Place stopper or marble on the test tubes and then place the entire rack in the boiling water bath. Cover the entire bath with foil and heat for 1 hr. Remove the rack, cool to room temperature and determine the absorbance at 540 nm against the 0 urea standard.

Make a standard curve and determine the urea concentration of both the 'blank' and 'test' assay samples. Enzyme activity is determined by the following equation:

Activity = $(\Delta U \times 0.4 \times 6) / (0.2 \times \text{mg protein [or g tissue] / assay})$.

Where:

ΔU = difference in urea concentration between the 'test' and the 'blank'

0.4 = final volume of the assay

6 = conversion from 10 min to 1 hr

0.2 = volume of the supernatant taken for determination

Argininosuccinate Lyase IsolationReagents1 M K_2HPO_4 , pH 7.5

K_2HPO_4	87.09 g
6 N HCl	± 5.0 mL
d-d- H_2O , q.s.	500 mL

Dissolve K_2HPO_4 in approximately 490 mL water and add HCl. Take 1 mL of the solution, dilute it to 10 mL (to make a 0.1 M solution) and test the pH. Make necessary pH adjustments, testing the pH at a 0.1 M concentration.

0.1 M K_2HPO_4 , pH 7.5

Take 100 mL of 1 M solution, dilute to 1000 mL and test the pH directly.

0.02 M K_2HPO_4 , pH 7.5

Take 40 mL of 1 M solution, dilute to 800 mL, adjust the pH if necessary, then dilute to 2000 mL.

0.02 M K_2HPO_4 , pH 7.0

Take 40 mL of 1 M solution, dilute to 800 mL, adjust the pH to 7.0 with 1 to 2 drops of 20% NaOH, then dilute to 2000 mL.

2 M Na-acetate, pH 4.6

Na-acetate	32.80 g
6 N HCl	± 8 mL
d-d- H_2O , q.s.	200 mL

Dissolve Na-acetate in approximately 180 mL water and add HCl. Test pH by taking 0.5 mL of Na-acetate solution and diluting it to 10 mL, make any necessary adjustments and retest pH if needed. Bring solution to 200 mL mark with water.

0.04 M Na-acetate, pH 5.0

Take 8 mL of 2 M solution and dilute to 160 mL, adjust pH with 20% NaOH, then dilute to 400 mL.

Equipment

centrifuge bottles
cold room (4°C)

Refrigerated Centrifuge
1 gal commercial blender

Isolation Procedure

Note that the isolation procedure may be halted overnight at the end of any step. Stopping prior to that, may result in inactivation of the enzyme. All steps are carried out in a cold room at approximately 4°C.

Step 1.

A. Obtain steer liver from a freshly slaughtered animal. Cut 1 Kg liver into 1-2 inch cubes and place in 1 gallon blender. Add 2 L 0.02 M K_2HPO_4 , pH 7.0 and homogenize at top speed for 45 sec.

B. Filter homogenate through cheesecloth and adjust the homogenate to pH 5.4 with approximately 112 mL 2 M Na-acetate, pH 4.6.

C. Centrifuge (Dupont Sorvall RC-5 Superspeed Refrigerated Centrifuge, Dupont Instruments, Willmington, DE) at 4°C at 16,000 x g for 20 min.

D. Adjust the supernatant to pH 7.0 with 1 M K_2HPO_4 , pH 7.5. While continuously stirring, add 400 g $(NH_4)_2SO_4$ over a 30 min period. Stir an additional 30 min.

E. Centrifuge at 16,000 x g for 20 min.

F. Dissolve the pellet in a minimal amount of 0.02 M K_2HPO_4 , pH 7.5.

G. Measure protein content of homogenate and adjust to 12 mg protein/mL with 0.02 M K_2HPO_4 , pH 7.5. Protein concentration is measured by the Lowry procedure.

H. Add 141 g $(NH_4)_2SO_4$ slowly and stir 1.5 hr longer. Centrifuge at 16,000 x g for 30 min. Save the supernatant.

Step 2.

A. Add 70.6 g $(NH_4)_2SO_4$ slowly and continue stirring for 1.5 longer.

B. Centrifuge at 16,000 x g for 30 min.

C. Dissolve the pellet in a minimal amount of 0.02 M K_2HPO_4 , pH 7.5 (approximately 36 mL). Dilute further with 360 mL 0.04 M Na-acetate, pH 5.0.

Step 3.

A. Heat solution in a 65°C shaking water bath (Dubnoff Metabolic Shaking Incubator, CGA/Precision Scientific, Chicago, IL). The solution should reach 50°C within 4 min and remain at that temperature an additional 2 min.

B. Chill to 0°C in a -20°C dry ice and acetone bath.

C. Centrifuge at 16,000 x g for 40 min. Save the supernatant.

D. Add 41.3 mL 1 M K_2HPO_4 , pH 7.5 to the supernatant.

E. Add 57.7 g $(NH_4)_2SO_4$ slowly and stir an additional 1.5 hr.

F. Centrifuge at 16,000 x g for 40 min. Save the supernatant.

G. Add 28.5 g $(NH_4)_2SO_4$ slowly and stir an additional 1.5 hr.

H. Centrifuge at 16,000 x g for 50 min. Discard the supernatant.

I. Take pellet up in 7 to 8 mL 0.1 M K_2HPO_4 , pH 7.5.

Determination of Enzyme Activity

Reagents

0.01 M Argininosuccinate

argininosuccinate, barium salt	50.03 mg
d-d- H_2O	9.85 mL
0.1 M K_2SO_4	0.15 mL

Dissolve argininosuccinate in water and K_2SO_4 . Centrifuge at 1000 rpm to clarify.

ProcedureIncubation Mixture

2.0 mL 0.1 M K_2HPO_4 , pH 7.5
 8-12 U enzyme
 0.25 mL argininosuccinate
 d-d- H_2O to 3.0 mL

Blank

2.0 mL 0.1 M K_2HPO_4 , pH 7.5
 amount of enzyme assayed
 amount of water added to assay mixture

1. Prepare blanks and samples without argininosuccinate and keep on ice.
2. Warm to room temperature just prior to use.
3. Add argininosuccinate, mix and read at 240 nm within 30 sec of substrate addition and each 30 sec interval thereafter. The enzyme should be linear for 10 min; 3-4 min should be sufficient to determine activity.
4. Determine the temperature after the last absorption reading.
5. Plot absorption versus time to determine the change of absorption/min.

Specific Activity (1U = 1 μ mol product/hr at 38°C)

Activity = $(\Delta \text{Abs} \times 60 \times 3 \times 1.10 \times T_f) / 2.44 \times \text{mg protein}$

Where

$$T_f = R_2 = R_1^{(T_1 - T_2)}$$

$$R_1 = Q_{10} \text{ at } 38^\circ\text{C} = 2.25$$

$$T_1 = 38^\circ\text{C}$$

$$T_2 = \text{enzyme temp. at end of assay}$$

Ornithine Carbamoyl Transferase IsolationReagentsHomogenate Buffer, pH 7.2

KCl	22.36 g
MOPS	837.04 mg
EDTA	67.24 mg
d-d-H ₂ O, q.s.	2000 mL

Dissolve KCl, MOPS and EDTA in approximately 1800 mL water, adjust the pH to 7.2 with 20% NaOH and bring to volume with water. Three volumes of homogenate buffer per g liver are needed twice. Thus, for 1 Kg liver, make 6 L homogenate buffer.

Buffer A, pH 6.0

Na ₂ HPO ₄	28.4 g
EDTA	672.4 mg
d-d-H ₂ O, q.s.	2000 mL

Dissolve Na₂HPO₄ and EDTA in approximately 1800 mL water, adjust the pH with 6 N HCl and bring to volume with water. For 1 Kg liver, make 8 L of Buffer A.

1 M KOH

KOH	28.1 g
d-d-H ₂ O, q.s.	500 mL

Dissolve KOH in approximately 490 mL water, then bring to volume.

1% CTAB

5 g CTAB in 500 mL d-d-H₂O

Procedure

Isolation is carried out in the cold room and the equipment needed is the same as for OCT isolation. Again, the isolation procedure may be halted at the end of a step.

Step 1.

A. Obtain liver from a freshly slaughtered steer (University of Florida, Department of Animal Science) and cut 1 Kg into 1 inch cubes.

B. Homogenize the liver in 3 volumes of Homogenate Buffer at full speed for 45 sec.

C. Filter the homogenate through cheese cloth and adjust the pH to 7.0 with 1 M KOH.

D. Centrifuge at 11,750 x g for 35 min. Discard the supernatant.

E. Resuspend the pellet in the original volume of Homogenate Buffer in the centrifuge bottles. Shake by hand to suspend and centrifuge at 11,750 x g for 35 min. Discard the supernatant.

F. Dissolve the pellet in 0.55 volumes of Buffer A and 0.24 volumes of CTAB. Stir for 15 min. Centrifuge at 11,750 x g for 25 min. Discard the supernatant.

G. Assay the pelleted extract and dilute the pellet to 55 U/mL with Buffer A.

Step 2.

A. Add $(\text{NH}_4)_2\text{SO}_4$ to obtain a final concentration between 1.8 and 2.6 M. Stir an additional 30 min.

B. Centrifuge at 11,750 x g for 35 min and discard the supernatant.

C. Assay the pelleted extract and dilute the extract to 250 U/mL with Buffer A

Step 3.

A. Add $(\text{NH}_4)_2\text{SO}_4$ to obtain a concentration between 1.7 and 2.2 M. Stir an additional 30 min.

B. Centrifuge at 11,750 x g for 35 min and discard the supernatant.

C. Assay the pelleted extract and dilute the extract to 400 U/mL with Buffer A.

Step 4.

- A. Add ornithine-HCl to obtain a concentration of 5 mM.
- B. In 200 mL portions, heat to 65°C in a shaking water bath set to 75°C. Keep at 65°C for 1 min, then cool immediately in a -20°C bath.
- C. Filter through a Buchner funnel through glass fiber filters (Fisher Scientific).
- D. Wash with 0.2 x the initial volume of Buffer A.
- E. Add $(\text{NH}_4)_2\text{SO}_4$ to the filtrate plus wash to obtain a concentration of 2.2 M. Stir for 30 min.
- F. Centrifuge at 11,750 x g for 35 min. Discard the supernatant.
- G. Dilute to desired activity for AS assay.

AL Activity Determination (1U=1.0 μmol product/min at 37°C)Reagents16.8 mM Ornithine, pH 8.5283.3 mg Ornithine-HCl/100 mL d-d-H₂O16.8 mM $(\text{NH}_4)_2$ Carbamoyl-phosphate29.42 mg CP/10 mL d-d-H₂ODiethanolamine acetate, pH 8.5

Na-acetate	13.6 g
diethanolamine	536.4 mg
d-d-H ₂ O, q.s.	100 mL

Dissolve Na-acetate in approximately 90 mL water, add and dissolve diethanolamine and adjust the pH to 8.5. Bring to volume with water.

EDTA-BSA-Mercaptoethanol

EDTA	336.2 mg
BSA	50 mg
2-mercaptoethanol	8.8 mL
d-d-H ₂ O, q.s.	100 mL

Dissolve EDTA and BSA in approximately 80 mL water.
Add 2-mercaptoethanol and bring to volume with water.

0.5 N HClO₄

HClO ₄ (70%)	4.3 mL
d-d-H ₂ O, q.s.	100 mL

Dilute indicated volume of HClO₄ to 100 mL with water.

ProcedureAssay Mixture

16.8 mM ornithine	0.3 mL
diethanolamine acetate	0.3 mL
enzyme or citrulline std	0.1 mL

Procedure

1. Prepare a 37°C water bath.
2. Combine all reagents and enzyme (or standard) in a 3 mL test tube. Keep on ice.
3. Initiate reaction by the addition of 0.3 mL carbamoyl phosphate. Place test tube in 37°C water bath for exactly 10 min.
4. Terminate the reaction by the addition of 1.0 mL HClO₄. Centrifuge tubes at 1000 rpm for 5 min to pellet protein precipitate.

Determination of Citrulline Production

Determine the concentration of citrulline by the same procedure as that outlined for CPS activity determination.

Activity = that amount of enzyme which catalyzes the formation of 1.0 μmol citrulline in 1.0 min at 37°C.

Note: To determine the amount of ammonium sulfate to add at each step, after the first addition, measure the total

volume of the suspension just prior to centrifugation and recalculate to molarity based on the increase in volume from the previous concentration determination. Using the formula by Pogell and McGilvery, add the amount of ammonium sulfate necessary.

Lowry Protein Determination

Reagents

2% NaCO₃ in 0.1 N NaOH

NaCO ₃	20.0 g
NaOH	4.0 g
d-d-H ₂ O, q.s.	1000 mL

Dissolve NaCO₃ and NaOH in approximately 900 mL water, then bring to volume.

0.5% CuSO₄ in 1% Na-citrate

CuSO ₄ -5 H ₂ O	0.5 g
Na-citrate	1.0 g
d-d-H ₂ O, q.s.	100 mL

Dissolve CuSO₄ and Na-citrate in approximately 90 mL water, then bring to volume.

1 N Folin Phenol Reagent

Dilute the 2 N commercially available stock to 1 N.

BSA Standard

0.5 mg/mL; dissolve 5 mg BSA in water and bring volume to 10 mL.

Chromogenic Reagent

0.5% CuSO ₄	0.5 mL
2% NaCO ₃	25 mL

Mix these two reagents immediately before use in the indicated proportion as necessary for the number of samples to be analyzed.

Standard Curve

<u>Concentration (mg/mL)</u>	<u>μL BSA Standard</u>
0.0625	25
0.125	50
0.25	100
0.375	150

Add the indicated volumes to duplicate or triplicate test tubes, then add enough d-d-H₂O to bring total volume to 0.2 mL.

Procedure

1. Add Standard or upto 0.2 mL sample to each 3 mL test tube. The blank consists of 0.2 mL d-d-H₂O.
2. Add 1 mL Chromogenic Reagent to each test tube and vortex to mix. Incubate at room temperature 10 min.
3. Add 0.1 mL of 1 N Folin Phenol reagent to each tube and vortex to mix. Incubate at room temperature for 30 min.
4. Read absorbance at 750 nm.
5. Plot standard curve and determine the concentration of the samples from the curve.

Serum Urea Nitrogen (BUN)ReagentsBUN Acid

Thiosemicarbazide	120 mg
4-aminoantipyrine	380 mg
sulfuric acid (98%)	56 mL
phosphoric acid (85%)	45 mL
d-d-H ₂ O, q.s.	1000 mL

Dissolve thiosemicarbazide and 4-aminoantipyrine in approximately 600 mL water. Add sulfuric acid and mix, then add phosphoric acid and mix.

BUN Color

2,3-butanedione monoxime	1.2 g
d-d-H ₂ O, q.s.	100 mL

Dissolve 2,3-butanedione monoxime in approximately 90 mL water, then bring to volume.

0.2 N Sulfuric Acid

5.55 mL H₂SO₄ and bring to 1000 mL

Urea StandardsStock Standard

Dessicated urea crystals	1.0705 g
0.2 N sulfuric acid, q.s.	500 mL

Dissolve urea in 0.2 N sulfuric acid, then bring to volume.

Working Standard

<u>Final Concentration (mg%)</u>	<u>mL Stock Standard</u>
5	5
10	10
20	20
40	40
60	60
80	80

Procedure

1. Prepare a boiling water bath.
2. Put 5 mL BUN acid in each 7 mL Pyrex test tube.
3. Put 0.5 mL BUN color in each tube.
4. Put 20 μ L 0.2 N H_2SO_4 (for blank), standard or serum sample into tubes. Vortex to mix and cover test tubes with vented stoppers or marbles.
5. Place test tube rack in boiling water bath. Cover bath with foil and heat for 12 min.
6. Cool to room temperature and read absorbance at 540 nm against the 0.2 N H_2SO_4 blank.
7. Plot a standard curve and determine the sample concentrations.

Amino Acid Analysis

Analysis

Physiological Samples. Analyses were performed using a Beckman 119CL Amino Acid Analyzer (Beckman Instruments, Palo Alto, CA) equipped with an integrator (Hewlett Packard Model 3390A data system) and a single 6 x 460 mm column packed with Beckman W-3P resin. Ninhydrin and lithium hydroxide buffers were prepared or diluted according to the recommendations of Beckman Instruments. Commercially available Amino Acid Calibration Standards P-AN and P-B (Cal-Biochem) were diluted 1:25 v/v in 0.2 N lithium citrate buffer, pH 2.2. A standard curve was obtained from an aliquot of 100 μ L of the calibration standards. Alpha-amino β -guanidinopropionic acid (AGPA) in 0.2 N lithium citrate buffer, pH 2.2, was added as an internal standard as a 1:1 dilution of the plasma. An aliquot of 100 μ L of diluted plasma was injected onto the column. The buffer flow rate was 44.0 mL/hr and ninhydrin rate at 22.0 mL/hr. The column temperature was 40°C for the first 40 minutes of analysis, then 65°C for the remaining 4 hrs.

Hydrolysis Samples. Analyses were performed using the Beckman 119CL equipped with a 6 x 460 mm single-column packed with Beckman W-3 resin (hydrolysate column). The signal was recorded on a Hewlett Packard integrator. Ninhydrin and sodium citrate buffers were prepared according to Beckman specifications. One hundred microliters of 2500

nmol/mL norleucine were added as an internal standard to 20 μ L of undiluted sample and the entire sample was injected onto the column. Buffer and ninhydrin flow rates were 44.0 and 22.0 mL/hr, respectively. The column temperature was 50°C for the first 20 minutes, then 65°C for the remaining 70 minutes of analysis.

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BIOGRAPHICAL SKETCH

Linda Fay Moore was born in Fresno, California, where she lived with her parents and two sisters. She graduated from a local high school and obtained an Associate of Arts at Fresno City College. She earned her Bachelor of Science and Master of Science from the University of California, Davis, both in food science.

After obtaining her Master of Science, Linda was employed as a quality control manager by Ashley's, Inc., in El Paso, Texas. She terminated her employment in order to care for her son and soon-to-be infant. She was then employed by Jerky Treats, Inc., also of El Paso as a laboratory technician. After approximately two years of employment by Jerky Treats, Linda decided to return to school to earn a doctorate with a nutrition emphasis.

At the University of Florida, Linda was employed as a Chemist II for the first three years of her doctorate education. She received a General Foods Fellowship in 1986 and at that time became a full-time student.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.



Harry S. Sitren, Cochair
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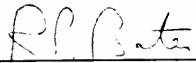
Rachel S. Shireman, Cochair
Professor of Food Science and
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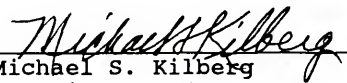
Peggy R. Borum
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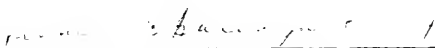
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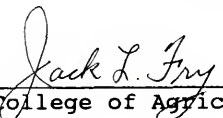
I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.



Thomas G. Baumgartner
Associate Professor of Nursing

This dissertation was submitted to the Graduate Faculty of the College of Agriculture and to the Graduate School and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

December 1989



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Dean, Graduate School

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